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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 9/127 — A1

(11) International Publication Number: WO 94/26253

(43) International Publication Date: 24 November 1994 (24.11.94)

(21) International Application Number: PCT/US94/05332

(22) International Filing Date: 16 May 1994 (16.05.94)

(30) Priority Data:
08/064,839 19 May 1993 (19.05.93) US
08/166,082 10 December 1993 (10.12.93) US

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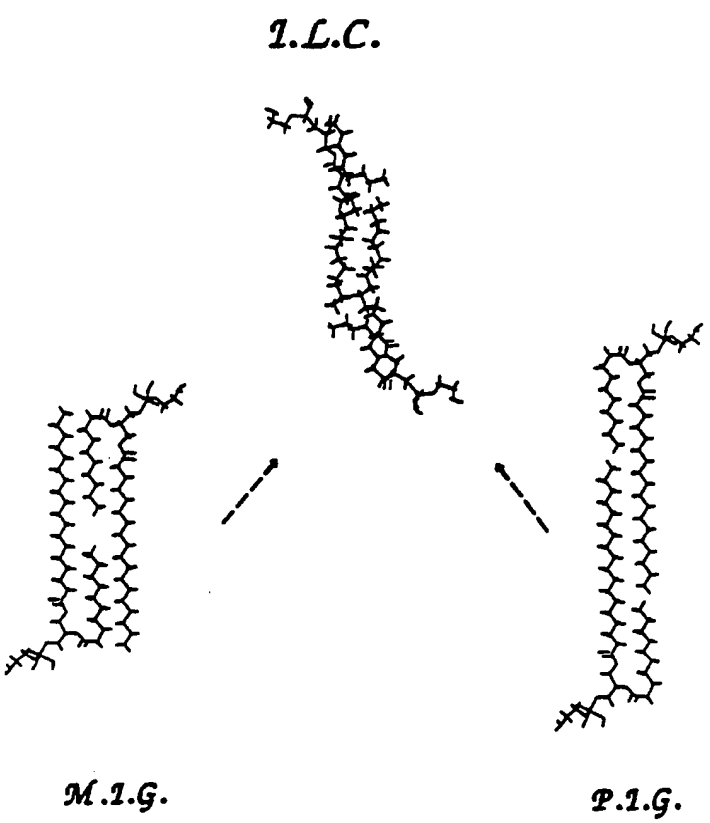
(81) Designated States: AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: LIPOSOME HAVING A MULTICOMPONENT BILAYER WHICH CONTAINS A BIOACTIVE AGENT AS AN INTEGRAL COMPONENT OF THE BILAYER

(57) Abstract

This invention provides a liposome having a multicomponent bilayer. This bilayer contains: a pocket-forming component which is an amphipathic lipid with asymmetric acyl chains, that is acyl chains of uneven length; a bilayer thickness component which is an amphipathic lipid with symmetric or asymmetric acyl chains, provided that any such asymmetry, or inequality of length, is less than or equal to the asymmetry of the acyl chains of the pocket-forming component; and a bioactive agent which is an integral component of the multicomponent bilayer. The multicomponent bilayer will, when necessary to inhibit phase separation of the pocket-forming and bilayer thickness components, also contain an amphipathic lipid known as a phase separation prevention component. The bioactive agent may be a therapeutic agent such as antibacterial, antifungal, antiviral and antiparasitic agents, and is likely to be an anticancer agent such as taxol, camptothecin or nogalamycin. Accordingly, pharmaceutical compositions containing the liposome of this invention may be administered to animals for the treatment or prevention of diseases such as antimicrobial infections and cancers.



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LIPOSOME HAVING A MULTICOMPONENT BILAYER WHICH CONTAINS A BIOACTIVE AGENT AS AN INTEGRAL COMPONENT OF THE BILAYER

Field of the Invention

This invention is directed to a liposome having a multicomponent bilayer containing a bioactive agent which is an integral component of the bilayer.

Background of the Invention

Liposomes are spontaneously self-assembling structures comprising one or more bilayers of amphipathic lipid molecules enclosing an internal aqueous volume. The amphipathic lipid molecules which make up the bilayer comprise a polar (hydrophilic) headgroup region covalently linked to one or more non-polar (hydrophobic) acyl chains. The energetically unfavorable contact between the hydrophobic acyl chains and the aqueous medium causes the molecules to rearrange such that the polar headgroups are facing the aqueous medium while the acyl chains reorient towards the interior of the bilayer. The net result is an energetically stable structure in which the acyl chains are effectively shielded from coming into contact with the aqueous medium.

The size distribution, lipid composition, lamellarity, as well as the other properties of presently available liposomes, may be tailored to meet specific needs. A variety of methods exist for producing liposomes (for a review, see, e.g., Szoka and Paphadjopoulos, in: Liposomes: From Physical Structure to Therapeutic Applications (C.G. Knight, ed., Elsevier/North Holland, pp. 51-82 (1981); Cullis et al., in: Liposomes, From Biophysics to Therapeutics M. J. Ostro, ed.), Marcel Dekker, pp. 39-72 (1987)). Bangham's original preparation (J. Mol. Biol. 13:238-252 (1965)) involves suspending phospholipids in an organic solution and then evaporating the solution to dryness, leaving a phospholipid film on the walls of the reaction vessel. Next, an appropriate amount of a chosen aqueous phase is added; the resulting liposomes are multilamellar vesicles (MLVs). This technique provided the basis for the development of sonicated unilamellar vesicles by Paphadjopoulos et al. (Biochem. Biophys. Acta. 135:624-638 (1968)). Lenk et al. (U.S. Patent Nos. 4,522,803, 5,030,453 and 5,169,637) and Fountain et al. (U.S. Patent No. 4,588,708) disclose methods for producing multilamellar liposomes with substantially equal interlamellar solute distribution. FATMLVs, freeze-and-thaw multilamellar vesicles, are disclosed by Cullis et al. in U.S. Patent No. 4,975,282. These vesicles are produced by first dispersing a lipid in an aqueous solvent to form multilamellar

liposomes. The resulting lipid vesicles are rapidly frozen, the frozen mixture is warmed, and then the freeze-thaw cycle is repeated at least three times. Furthermore, Janoff et al. (U.S. Patent No. 4,721,612) and Bolcsak et al. (U.S. Patent No. 5,100,662) describe the use of sterols for the preparation of liposomes having enhanced stability.

Liposomes can be loaded with bioactive agents passively, i.e., by solubilizing the molecule in the medium in which the liposomes are formed in the case of water-soluble agents or adding lipid-soluble agents to the lipid solutions from which the liposomes are made. Bioactive agents can also be loaded into liposomes actively, e.g., by establishing a potential gradient across the liposomal membrane and then adding the agent to the external medium (see Bally et al., U.S. Patent No. 5,077,056).

Bioactive molecules entrapped within liposomes can have an enhanced therapeutic index and improved biodistribution. Liposomal drugs are gradually released in the circulation, thereby alleviating the toxic side effects associated with administration of the free drug and minimizing the amount of the drug that need be administered to maintain desired serum levels. Additionally, drug-lipid formulations may be directed to intracellular sites of infection. However, preparation of liposomal formulations requires the development of vesicles capable of entrapping the drugs and preserving them in a useful form. As described above, known liposomes are useful in connection with lipid- and water-soluble agents. However, they are not well suited for the preparation of formulations of drugs poorly soluble in liposomal aqueous compartments or drugs which are not incorporated in a physically stable, pharmaceutically useful fashion into the hydrophobic bilayer interior.

In the lipid bilayers presently available, large hydrophobic molecules sequestering themselves within the bilayer interior increase acyl chain disorder as the lipid acyl chains readjust their packing as they attempt to form around the molecules whilst maintaining bilayer integrity. As more molecules are added, the resulting disorganization of the acyl chains reaches a point at which bilayer stability is compromised, either by the inability to maintain the optimal surface area at the headgroup/interfacial region or by the exposure of imperfectly packed acyl chains to the aqueous medium. Since lipid bilayers can only tolerate a limited degree of packing disorganization, excess hydrophobic molecules are ejected. This restores bilayer integrity, but also leads to crystal formation. Furthermore, no mechanism exists in an "ordinary" bilayer to

provide a "cage" or "fence" between neighboring molecules to reduce the likelihood of intermolecular collisions between hydrophobic molecules.

Huang and Mason (Biochim. Biophys. Acta. 864:423 (1986)) and Slater and Huang (Prog. Lipid Res.:325 (1988), and in: The Structure of Biological Membranes, CRC Press (1992), pp. 175-210) review the formation and structure of lipid bilayers formed with amphipathic lipids having acyl chains of unequal length, i.e., asymmetric lipids. However, none of these references recognize the use to which asymmetric lipids are put by this invention. This invention provides a liposome having a multicomponent bilayer incorporating a "relief valve" which compensates for the impending effects of acyl chain disorder as bioactive agent is added to the bilayer interior. This relief valve comprises an asymmetric lipid, which allows the bilayer to undergo a structural transformation between one organization in the absence of a bioactive agent to a distinct structural organization in its presence. These references note the different forms of interdigitated structure which lipids with asymmetric acyl chains can assume. However, none of these references teach or suggest that an asymmetric lipid can undergo a structural transformation from an interdigitated state in the absence of a bioactive agent to a noninterdigitated state in its presence, let alone that during this transformation, the bioactive agent can become an integral component of a bilayer of which the asymmetric lipid is a component. None of the available references teach or suggest that individual bioactive agents are shielded from coming into contact with each other by the acyl chains of multicomponent bilayers having an asymmetric lipid, which pack around individual bioactive agent molecules and shield them from their nearest neighbors.

One bioactive agent useful in accordance with the practice of this invention is taxol (see NCI Investigational Drugs (Chemical Information), NIH Publication No. 88-2654 (August, 1988), pp. 94-97 and Schiff and Horwitz, Proc. Natl. Acad. Sci. USA 77(3):1561-1565 (1980)). Taxol is an antimitotic agent which binds to tubulin, blocking disassembly of microtubules and thereby, cell division (Schiff et al., Nature 277:665 (1979)). The optimal effect of taxol on polymerization and stabilization of microtubules is seen at concentrations near stoichiometric equivalence with tubulin dimers (Schiff and Horowitz, Proc. Natl. Acad. Sci. USA 77(3):1561-1565 (1980)). Taxol has been found to have activity against ovarian and breast cancers in particular, as well as against other cancers such as malignant melanoma, colon cancer, leukemias and lung cancer

(see, e.g., Borman, Chemical & Engineering News, September 2, 1991, pp. 11-18; The Pharmacological Basis of Therapeutics (Goodman Gilman et al., eds.), Pergamon Press, New York (1990), p. 1239; Suffness, Antitumor Alkaloids, in: "The Alkaloids, Vol. XXV," Academic Press, Inc. (1985), Chapter 1, pp. 6-18; Rizzo et al., J. Pharm. & Biomed. Anal. 8(2):159-164 (1990); and Biotechnology 9:933-938 (October, 1991).

Taxol, derived from the bark of the Pacific yew Taxus brevifolia, is highly insoluble in water and aqueous solvents, thus limiting its bioavailability when administered to animals in the free form and making preparation of liposomal formulations difficult. The drug is currently supplied as a concentrated sterile emulsion of 6 mg taxol per ml of a 50:50 mixture of CremophorEL™ (Bristol Myers Squibb), which is a combination of a polyoxyethylated derivative of castor oil and ethanol. The concentrated emulsion is diluted to 0.6 mg/ml, for therapeutic use, with sterile sodium chloride or dextrose solutions. Administration of this formulation entails premedication with other drugs and a slow infusion of a large volume over 24 hours to avoid toxicity associated with the cremophor vehicle, thereby requiring that patients receiving taxol be admitted to hospitals over night. Additionally, because of the poor solubility of taxol in aqueous solutions, care must be exercised that the taxol does not precipitate from solution.

Summary of the Invention

This invention provides a liposome having a multicomponent bilayer comprising: a first amphipathic lipid which comprises an acyl chain of length A and an acyl chain of length B, wherein A and B are integers equal to the numbers of carbon atoms in the acyl chains and A is greater than B; a second amphipathic lipid which comprises an acyl chain of length C and an acyl chain of length D, wherein C and D are integers equal to the numbers of carbon atoms in the acyl chains and C is greater than or equal to D; and a bioactive agent, wherein the difference between A and B is preferably greater than, but may be equal to, the difference between C and D, and wherein the bioactive agent is an integral component of the bilayer. The first amphipathic lipid is known as the pocket-forming component of the multicomponent bilayer; the second amphipathic lipid is known as the bilayer thickness component. In preferred embodiments of this invention, "A" equals "C" and "C" equals "D". The first and

second amphipathic lipids are preferably phospholipids, most preferably, phosphatidylcholines.

The multicomponent bilayer of the liposome of this invention, when necessary to prevent lateral phase separation of the pocket forming and bilayer thickness components, may further comprise a phase separation prevention component which comprises an amphipathic lipid.

In one embodiment of this invention, the first and second amphipathic lipids comprise a single bipolar lipid, that is, a lipid with two polar headgroups, each of which is linked to the same bilayer-spanning acyl chain and both of which are separately linked to shorter acyl chains, which together do not span the entire width of the bilayer.

The bioactive agent may be a therapeutic agent, e.g., an anticancer, antifungal, antibacterial, antiviral, antiparasitic, anti-aging, anti-inflammatory or growth-promoting agent. Preferably, the therapeutic agent is an anticancer agent, more preferably, taxol, camptothecin or nogalamycin and most presently preferable, taxol. The liposome of this invention may further comprise a second bioactive agent, which may also be an integral component of the multicomponent bilayer, or which may be otherwise entrapped in the liposome. The liposome may also further comprising an amount of a cyclodextrin effective to inhibit crystallization of the bioactive agent. The cyclodextrin is preferably a chemically modified cyclodextrin, e.g., hydroxypropyl gamma cyclodextrin.

The liposome of this invention may be dehydrated, according to known procedures, to allow it to be stored and thereby extend its shelf life.

Presently, it is preferred that the first amphipathic lipid, the pocket forming component, is C(18):C(10)-PC (SCPC). When the first amphipathic lipid is SCPC, the second amphipathic lipid is preferably C(18:1):C(18:1)-PC (DEPC) and the multicomponent bilayer of the liposome further comprises a phase separation prevention component which comprises an amphipathic lipid. Desirably, the phase separation prevention component comprises C(14):C(14)-PC (DMPC). In presently preferred embodiments of this invention, the bioactive agent is an anticancer agent, more preferably taxol, camptothecin or nogalamycin and most preferably, taxol.

Also provided is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and the liposome of this invention. This pharmaceutical composition may be used in a method of treating an animal,

e.g., a mammal, and preferably a human, afflicted with a disease which comprises administering to the animal a therapeutically effective amount of the pharmaceutical composition.

In a presently preferred embodiment of this invention, the bioactive agent incorporated into the multicomponent bilayer of the liposome is an anticancer agent. Accordingly, the preferred uses for the liposome and pharmaceutical composition provided herein are the treatment of animals afflicted with a cancer, e.g., brain, ovarian, lung, colon or breast cancer. In preferred embodiments of this invention, the disease treated is a cancer and the liposome comprises a multicomponent bilayer comprising DEPC, DMPC, SCPC, and taxol or camptothecin. However, the liposome and pharmaceutical composition may also be used to treat diseases caused by bacterial, viral, fungal or parasitic infections, or due to steroidal, regenerative, growth-associated or other diseases, disorders or conditions.

Further provided is a unit dosage form of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the liposome of this invention. Typically, the therapeutically effective amount of the liposome is from about 1 milligram of the liposome per kg of the body weight of the animal to which the pharmaceutical composition comprising the liposome is administered to about 1000 milligrams per kg, desirably from about 100 milligrams per kg of body weight to about 400 mg per kg. The ratio of bioactive agent to lipid in the liposome is typically from about 1:50 to about 1:5, but may be made higher or lower when it is necessary to do so.

Brief Description of the Drawings

Figure 1. Depiction of the non-interdigitated configuration of a lipid (SCPC/(C(18:C(10)-PC)) with acyl chains of unequal length.

Figure 2. Thermotropic behavior of SCPC as a single component system. This lipid undergoes a transition from the mixed interdigitated gel (M.I.G.) state to the interdigitated liquid crystalline (I.L.C.) phase. As a single component system, it never exhibits a partially interdigitated (P.I.G.) phase.

Figure 3. Chemical structures of hydrophobic molecules incorporated into the multicomponent liposome. A: taxol; B: nogalamycin; C: itraconazole (Janssen R51211); D: camptothecin; E: pregnenolone.

Figure 4. Three-dimensional graphical representations of results derived from differential scanning calorimetry studies of a liposome having a multicomponent bilayer comprising SCPC, DEPC and DMPC. The base of the cube contains the triangular three-component phase diagram plotted in the x-y plane. The vertical (z) axis can represent any single experimental measurement; for these figures, the transition onset temperatures is plotted on the z axis. A three-dimensional surface can be drawn through these data. A minimum in the onset temperature corresponds with the optimal proportions of SCPC, DEPC and DMPC in the multicomponent bilayer (SCPC: 0.300; DEPC: 0.375; DMPC: 0.325). As = lipid with asymmetric acyl chains; DM = dimyristoyl phosphatidylcholine (DMPC); DE = dielaidoyl phosphatidylcholine (DEPC). A-G: different views of the three-dimensional plot.

Figure 5. Differential scanning calorimetry (DSC) results for a multicomponent bilayer comprising SCPC, DEPC, DMPC and taxol. Samples of this ternary lipid system were prepared at target taxol:lipid ratios of 0, 10, 20, 30, 40, 50, 60, 70, 80 micrograms taxol per milligram lipid

Figure 6. Schematic depictions of a bipolar lipid. A and B: mirror image views of a bipolar lipid in which two polar headgroups are both esterified to the same bilayer-spanning acyl chain. Each of the headgroups is linked to a separate, shorter acyl chain. The combined lengths of these shorter acyl chains does not equal the length of the membrane-spanning acyl chain.

Detailed Description of the Invention

The following abbreviations are used throughout this application: DMPC: dimyristoyl phosphatidylcholine; DPPC: dipalmitoyl phosphatidylcholine; DSPC: distearoyl phosphatidylcholine; DMPG: dimyristoyl phosphatidylglycerol; POPG: 1-palmitoyl-2-oleoyl-phosphatidylglycerol; POPC; 1-palmitoyl-2-oleoyl-phosphatidylcholine; SOPC: 1-stearoyl-2-oleoyl-phosphatidylcholine; DOPC: dioleoyl phosphatidylcholine; DEPC: dielaidoyl phosphatidylcholine; BPS:EPC: brain phosphatidylserine: egg phosphatidylcholine; EPC: egg phosphatidylcholine; CHS: cholesteryl hemisuccinate; THS: tocopherol hemisuccinate; MOPE: mono-oleoyl phosphatidylethanolamine; DPhytPC: diphytanoyl phosphatidylcholine; DLPE: dilauroyl phosphatidylethanolamine; DOPE: dioleoyl

phosphatidylethanolamine; and SCPC: 1-stearoyl-2-capryl-3-sn-phosphatidylcholine.

Liposomes are spontaneously self-assembling structures comprising one or more lipid bilayers surrounding an internal aqueous volume. Lipid bilayers comprise two opposing monolayers of amphipathic lipid molecules, each of which comprises a polar (hydrophilic) headgroup adjacent to an internal or external aqueous phase and hydrophobic acyl chains arrayed in the bilayer interior. The formation of stable bilayers reflects an energy balance of hydrophobic effects from the interaction of acyl chains and the surrounding aqueous environment, steric packing constraints on the acyl chains, attractive and repulsive interactions at the interface of the bilayer with the aqueous environment, curvature elasticity of the bilayer, and the like.

Liposomes may have one lipid bilayer, i.e., they may be unilamellar vesicles, or multiple bilayers, i.e., they may be multilamellar vesicles (MLVs). Unilamellar vesicles may be small (SUVs) or large unilamellar vesicles (LUVs). LUVs are liposomes with average diameters of from about 50 nm to about 200 nm. MLVs may be prepared by dissolving lipids in an organic solvent, evaporating the solvent and then adding an aqueous medium to the resultant lipid film (see, e.g., Bangham, J. Mol. Biol. 13:238 (1965)). Freeze-and-thaw multilamellar vesicles (FATMLVs) are prepared by the repeated freezing and thawing of multilamellar liposomes according to the procedure described in Cullis et al. (U.S. Patent No. 4,975,282). Lenk et al. (U.S. Patent Nos. 4,522,803, 5,030,453 and 5,169,637) and Fountain et al. (U.S. Patent No. 4,588,708) disclose methods for producing multilamellar liposomes with substantially equal interlamellar solute distribution. LUVs may be prepared by extruding MLVs, under pressure, through filters with defined pore sizes. The filter pore size is adjusted according to the size of LUV desired (see Cullis et al., U.S. Patent No. 4,588,708 and Loughrey et al., U.S. Patent No. 5,059,421). The contents of these references are incorporated herein by reference to describe the state of the art with respect to liposome preparation.

Amphipathic lipids, used in conjunction with the above-described methods to prepare liposomes, comprise a polar (hydrophilic) headgroup and one or two hydrophobic acyl chains. The headgroups may be phosphate, sulfate, amino or other suitable polar moities; the acyl chains may be between 1 and 24, or more, carbon atoms in length and may have one or more double bonds, i.e., may be saturated or unsaturated. Preferably, the amphipathic lipids used in

accordance with the practice of this invention are phospholipids, most preferably, phosphatidylcholines.

This invention provides a liposome having a multicomponent bilayer comprising: a first amphipathic lipid which comprises an acyl chain of length A and an acyl chain of length B, wherein A and B are integers equal to the numbers of carbon atoms in the acyl chains and A is greater than B; a second amphipathic lipid which comprises an acyl chain of length C and an acyl chain of length D, wherein C and D are integers equal to the numbers of carbon atoms in the acyl chains and C is greater than or equal to D; and a bioactive agent, wherein the difference between A and B is greater than or equal to the difference between C and D and wherein the bioactive agent is an integral component of the bilayer.

"Multicomponent" as used herein describes lipid bilayers comprising a bioactive agent, a first amphipathic lipid denoted the pocket forming component and a second amphipathic lipid denoted the bilayer thickness component. Multicomponent bilayers of the liposomes of this invention may further comprise a phase separation prevention component which comprises an amphipathic lipid. Accordingly, a multicomponent bilayer of a liposome of this invention has more than one component.

"Bioactive agent," as used herein, means a chemical compound, whether synthetically produced or naturally derived, that exhibits biological activity. The terms "bioactive agent" and "bioactive agent molecules" are both used to denote such compounds. Bioactive agents suitable for use in accordance with the practice of this invention include, but are not limited to: antiviral, antibacterial, antifungal, antiparasitic or tumoricidial compounds, sterols, proteins, dyes, toxins, enzymes, immunomodulators, immunoglobulins, hormones, neurotransmitters, glycoproteins, radiolabels, radiopaque compounds, fluorescent compounds, cell receptor proteins, cell receptor ligands, antiinflammatory compounds, antiglaucomic agents, mydriatic compounds, bronchodilators, local anaesthetics, growth promoting agents and regenerative agents.

The first amphipathic lipid of the multicomponent bilayer is its pocket forming component. "Pocket forming component" as used herein describes an amphipathic lipid with asymmetric acyl chains, that is, acyl chains with an unequal number of carbon atoms and hence, of uneven length. "A" denotes the longer acyl chain of the first amphipathic lipid, or pocket forming component,

and "B," the shorter acyl chain. The asymmetry of acyl chain length allows the pocket forming component to undergo a change in configuration, from an interdigitated state in the absence of a bioactive agent to a non-interdigitated state in its presence. The pocket thereby created in the bilayer is filled by the bioactive agent which induces its formation.

The second amphipathic lipid of the multicomponent bilayer is its bilayer thickness component, with "C" and "D" denoting the lengths of its acyl chains. It is preferred, but not required, that the acyl chains of this component be of equal, symmetric, length, i.e., that "C" equals "D." However, when the acyl chains are of unequal length, "C" is used to denote the longer, and "D" the shorter, acyl chain. "Bilayer thickness component" as used herein denotes an amphipathic lipid that defines the thickness of the multicomponent bilayer. "Defining thickness" as used herein means that the thickness of the multicomponent bilayer will be approximately the same as the thickness of a bilayer containing the bilayer thickness component, but not the other component(s). Except for the terminal methyl gap, the space between the methyl terminal ends of acyl chains in opposing monolayers normally found at the bilayer midplane, the acyl chains of the bilayer thickness component will span the entire width of the bilayer interior.

It is preferred that the longer acyl chains of the first and second amphipathic lipids be of equal length, i.e, that "A" equals "C." However, "C" may also be greater than "A". The critical feature of the bilayer thickness component is that it comprise one, if not two, acyl chains of a length sufficient to induce the pocket forming component's acyl chains to adopt a non-interdigitated packing conformation in the presence of a bioactive agent, i.e., that the acyl chains do not cross the bilayer midplane and interact with acyl chains of a pocket forming component in the opposing monolayer. In ordinary bilayers, the acyl chains of the amphipathic lipids of opposing monolayers do not normally cross the bilayer midplane. Bilayers comprising interdigitated amphipathic lipids have acyl chains which cross the bilayer midplane and interact with acyl chains in the opposing monolayer.

The other acyl chain of the second amphipathic lipid, i.e, the acyl chain of length "D," is preferably longer than the shorter acyl chain of the first amphipathic lipid, i.e., the acyl chain of length "B". Accordingly, it is preferred that the difference between "A" and "B" is greater than the difference between "C" and "D," i.e., that the first amphipathic lipid have more asymmetry than the

second. However, "D" and "B" may also be equal. Thus, it is within the teaching of this invention that the first and second amphipathic lipid comprise acyl chains of the same lengths, i.e., that "A" equals "C" and "B" equals "D." Accordingly, when the headgroup of the first and second amphipathic lipids is the same, the first and second amphipathic lipid is the same lipid.

In one embodiment of this invention, the first and second amphipathic lipids may comprise a single, bipolar lipid. A "bipolar lipid" (see Figure 6) comprises two polar headgroups, both of which are linked to an acyl chain which spans the entire width of the bilayer interior (the "bilayer-spanning" acyl chain). This acyl chain has about twice the length of the longest acyl chain of the bilayer thickness component. Bipolar lipids form bilayers, in the presence of a bioactive agent, whose thickness is defined by the length of the bilayer-spanning acyl chain. This thickness is about the same as the thickness of a bilayer formed from amphipathic lipid molecules having symmetric acyl chains, each of which has a length equal to one half of that of the bilayer-spanning chain (taking into account the terminal methyl gap normally found between the terminal methyl groups of opposing acyl chains in ordinary bilayers, but not found in bilayers made from bipolar lipids). Each polar headgroup is also linked to a second acyl chain, this second acyl chain not being linked to the other headgroup. The total number of carbon atoms in both of these two second acyl chains is less than the number of carbon atoms in the bilayer-spanning acyl chain. Accordingly, bipolar lipids have acyl chains of unequal, asymmetric, length, which can lead to formation of a pocket in the presence of a bioactive agent. Thus, the two shorter acyl chains of a bipolar lipid fill the role of the pocket forming component. Bipolar lipids are preferably formed by: preparing a longer acyl chain of the desired langth, i.e., having a number of carbon atoms equal to "A" plus "C", this longer acyl chain defining the thickness of bilayers comprising the bipolar lipid; selecting two shorter acyl chains such that their combined size is shorter than the length of the longer acyl chain, thereby establishing the degree of asymmetry desired for the bioactive agent selected to be an integral component of the bilayer; and attaching the longer acyl chain to two separate headgroups while linking each of the shorter acyl chains to one of the headgroups.

While the liposome of this invention is preferably a large unilamellar vesicle and the above-described extrusion technique is the preferred method of its preparation, the liposome of this invention is not limited to being prepared

by any of the above-described techniques. Rather, the liposome may be made by any of those techniques presently available for producing liposomes.

Bioactive agents preferred for use in accordance with the practice of this invention are those chemical compounds having structures which limit their solubility in aqueous media, and which are poorly soluble in lipids. Presently, it is preferred that such a bioactive agent is a therapeutic agent, i.e., an agent capable of treating or preventing diseases or disorders in living organisms to which it is administered. Therapeutic agents may be anticancer, antibacterial, antiviral, antifungal, antiparasitic, antiaging, antiinflammatory, growth promoting or other such agents capable of treating or preventing diseases, disorders or conditions.

Preferably, the therapeutic agent in the liposome of this invention is an anticancer agent, e.g., taxol, camptothecin or nogalamycin. Most preferably, the anticancer agent is taxol. "Taxol" as used herein is meant to include the compound itself ($C_{47}H_{51}NO_{14}$, tax-11-en-9-one (see Figure 3)) as well as taxol analogs, i.e., those compounds, whether synthetically produced or naturally derived, with similar structures and activities. Taxotère, which differs from taxol by having a *tert*-butoxycarbonyl group instead of a benzoyl group on the C-13 side chain, and a hydroxyl group instead of an acetoxyl group at C-10, is one such analog (see, e.g., Borman, *supra*). Other analogs include those which are derivatives of a hydroxylated taxane compound found in yew needles, e.g., baccatin-based 14-hydroxy taxol analogs such as 14-hydroxy-10-deacetylbaccatin III (see, e.g., Borman, Chemical and Engineering News, April 12, 1993, pp. 36-37). The liposome may further comprise a second bioactive agent, which may also be an integral component of the bilayer. Alternatively, the second bioactive agent may be entrapped in an aqueous compartment of the liposome.

The multicomponent bilayer provides a pocket into which such a bioactive agent may fit without disrupting acyl chain packing. In "ordinary bilayers," i.e., those without pocket-forming and bilayer thickness components, insufficient space exists to accomodate the preferred bioactive agents without incurring disruption of acyl chain packing. Each bioactive agent molecule which sequesters itself within the bilayer, due to its hydrophobic nature, causes an increasing amount of acyl chain disorder as the chains readjust in their attempt to pack around the molecule. Disorder in the bilayer may eventually reach the point at which stability is compromised. Restoration of bilayer stability may

result in the ejection of an amount of the bioactive agent sufficient to lower chain disorder below the critical point. These "excess" molecules, i.e., the amount of molecules greater than that which can be tolerated by a stable bilayer, form crystals or other aggregates of minimal therapeutic utility.

The pocket forming and bilayer thickness components enable the bioactive agent to become an integral component of the multicomponent bilayer. The pocket formed by addition of the bioactive agent to a mixture of the pocket forming and bilayer thickness components minimizes the increase in acyl chain disorder, and may actually decrease it. Minimizing the amount of disorder allows for more bioactive agent to be incorporated into the multicomponent bilayer than can be incorporated into ordinary bilayers. Up to one bioactive agent molecule may be incorporated into the multicomponent bilayer for each pocket forming component for which there is an available bilayer thickness component.

"Integral" describes a bioactive agent which induces a change in conformation of the pocket forming component, in the presence of the bilayer thickness component, from an interdigitated to noninterdigitated state, and which is incorporated into the pocket in the bilayer thereby created. "Interdigitation" and "interdigitated" are used herein to denote lipid bilayers in which the acyl chains of the pocket forming component in each monolayer cross the bilayer midplane and penetrate into the opposing monolayer, where they interact with the asymmetric acyl chains of another pocket forming component. Lipids may be fully, mixed or partially interdigitated. Full interdigitation describes lipid bilayers in which each acyl chain in the bilayer spans the entire width of the bilayer, i.e., where there are four acyl chains per headgroup surface area. Thus, asymmetric lipids cannot be fully interdigitated. Mixed interdigitation describes the association of lipids with asymmetric acyl chains where the longer acyl chains span the entire width of the bilayer interior and the shorter acyl chains meet end-to-end across the gap between their terminal methyl groups, i.e., the "terminal methyl gap." There are three acyl chains per headgroup surface area when two asymmetric lipids combine in a mixed interdigitated conformation. Partially interdigitated asymmetric lipids have two acyl chains per headgroup surface area, with the longer acyl chain of one lipid meeting end-to-end with the shorter acyl chain of the other across the gap between their terminal methyl groups. In the case of noninterdigitated lipids, their acyl chains do not ordinarily cross the bilayer midplane.

Optimal incorporation of bioactive agent into the multicomponent bilayer occurs when the pocket forming and bilayer thickness components are combined in proportions such that a eutectic combination is formed. The components are most miscible with each other when they are combined in eutectic proportions. Maximal miscibility means that the maximum amount of the bilayer thickness component will be available to aid in transformation of the pocket forming component from an interdigitated to non-interdigitated state. As discussed hereinabove, the bioactive agents fits into the bilayer pocket created when its addition induces the pocket forming component to undergo this conformational transformation. Thus, when the bilayer thickness and pocket forming components are combined to form a eutectic composition, the maximum amount of bioactive agent, one molecule of the agent per pocket forming component, may be incorporated into the multicomponent bilayer. The relative proportions of the components may be altered such that they do not form a eutectic composition, but nevertheless form a stable bilayer in the presence of a bioactive agent. Sub-maximum amounts of bioactive agents will be incorporated into these noneutectic bilayers. The eutectic composition of a combination of amphipathic lipids can readily be determined by ordinarily skilled artisans using differential scanning calorimetry (DSC) analysis of the liquid/gel phase behavior of mixtures of different proportions of the lipids. The eutectic composition of a combination of amphipathic lipids is the point at which a bilayer containing the lipids has a lower gel-liquid transition temperature than does a bilayer formed from any of the individual lipids.

Raman spectroscopy can be used by ordinarily skilled artisans to determine the most probable mode of acyl chain packing of a combination of amphipathic lipids. Pocket forming and bilayer thickness components will be misicble when they adopt compatible acyl chain packing. When they do not, the components may exhibit lateral phase separation when combined. Formation of multicomponent bilayers in such cases requires the presence of a third amphipathic lipid, the "phase separation prevention component." This is an amphipathic lipid with symmetric or asymmetric, saturated or unsaturated, acyl chains that inhibits or prevents lateral phase separation of two or more amphipathic lipids by adopting compatible acyl chain packing with each lipid.

The pocket forming component comprises acyl chains of lengths "A" and "B", where "A" and "B" are asymmetric, i.e., unequal. Presently, it is preferred that "A" is equal to 18, "B" is equal to 10 and the amphipathic lipid is a

phosphatidylcholine, i.e., that the pocket forming component is C(18):C(10)-PC (1-myristoyl-2-capryl-3-sn-phosphatidylcholine (SCPC)). The bilayer thickness component comprises an amphipathic lipid with acyl chains of lengths "C" and "D". "C" is preferably, but not necessarily, equal to "A". Accordingly, when the pocket forming component is C(18):C(10)-PC, it is presently preferred that the bilayer thickness component is C(18:1):C(18:1)-PC (1-elaidoyl-2-elaidoyl-3-sn-phosphatidylcholine (DEPC)).

SCPC and DEPC exhibit lateral phase separation as a binary system because of their inability to adopt compatible acyl chain packing. Accordingly, formation of SCPC/DEPC multicomponent bilayers requires the presence of a phase separation prevention component. Presently, it is preferred that this component is C(14):C(14)-PC (1-myristoyl-2-myristoyl-3-sn-phosphatidylcholine (DMPC)). The phase behaviors of SCPC/DMPC and DEPC/DMPC binary systems have been characterized. DMPC and DEPC form a eutectic composition at 25 mole percent DMPC (see van Dijck et al., Biochim. Biophys. Acta 470 (1977)). SCPC and DMPC form eutectic compositions when the relative proportion of DMPC in the mixture is 40 mole percent (see Lin and Huang, Biochim. Biophys. Acta 946: 178 (1988)). The SCPC/DMPC and DEPC/DMPC binary systems can be combined to form a ternary lipid mixture in which the acyl chain packing incompatibilities between SCPC and DEPC are overcome by the favorable interactions of both with DMPC. Combination of eutectic binary systems in equal proportions results in a ternary lipid system comprising 37.5 mole percent DEPC, 32.5 mole percent DMPC and 30.0 mole percent SCPC. Accordingly, these are the optimal proportions of SCPC, DEPC and DMPC to be used in the multicomponent bilayer. However, SCPC, DMPC and DEPC may also be combined in proportions such that a eutectic composition is not formed, but a stable multicomponent bilayer is. This bilayer will be able to incorporate less of the bioactive agent than can a bilayer in which the phospholipids are combined in eutectic proportions.

Presently, it is preferred that the bioactive agent that is an integral component of a SCPC/DEPC/DMPC multicomponent bilayer be an anticancer agent, e.g., taxol, camptothecin or nogalamycin. Most preferably, the anticancer agent is taxol. The liposome comprising this multicomponent bilayer may further comprise a second bioactive agent, which may be an integral component of the bilayer, but may also be otherwise entrapped in the liposome.

Bioactive agents of differing dimensions may be incorporated into the multicomponent bilayer by adjusting the acyl chain lengths of the pocket forming component, i.e., adjusting its asymmetry, to fit the bioactive agent. The bilayer thickness component should constrain the pocket forming component to adopt a non-interdigitated conformation in the presence of the bioactive agent. The thickness of a bilayer containing the bilayer thickness component, but not the other components, will be approximately equal to the thickness of a bilayer formed from an amphipathic lipid having symmetrical acyl chains, each having a length about equal to that of the longer acyl chain of the pocket forming component (i.e., the acyl chain of length A of the first amphipathic lipid). These bilayer thicknesses can be measured by x-ray diffraction or any other technique known in the art suitable for measuring bilayer thicknesses. The phase separation prevention component, if necessary, should force the pocket forming and bilayer thickness components to adopt compatible acyl chain packing, i.e., that pocket forming and bilayer thickness components be able to pack next to each other in non-interdigitated configurations. The most probable mode of acyl chain packing can be determined by Raman spectroscopy or any other suitable technique. The length of the acyl chains of the phase separation prevention component are such that a bilayer formed from it, without the other components, is approximately equal to the thickness of a bilayer formed from the pocket forming component arrayed in a partially interdigitated conformation.

The multicomponent liposome of this invention may be lyophilized, stored and rehydrated by known procedures (see, e.g., Janoff et al., U.S. Patent No. 4,880,635, the disclosure of which is incorporated herein by reference).

This invention provides a liposome comprising an amount of a cyclodextrin effective to enhance the solubility of, and thereby inhibit crystallization of, the bioactive agent. Accordingly, the invention provides a liposome in which the bioactive agent is both an integral component of the bilayer and is otherwise entrapped in the liposome. This latter form of the bioactive agent may be associated with a cyclodextrin to enhance its solubility. The cyclodextrin may be the parent compound, but is preferably, a chemically modified cyclodextrin. Naturally occurring cyclodextrins (CDs) alpha, beta and gamma are cyclic polymers with 6,7 or 8 glucopyranose units. These form a three-dimensional "donut-shaped" structure. The interior of the cyclodextrin molecule is hydrophobic while the exterior is hydrophilic. These features allow

cyclodextrins to form inclusion complexes which enhance the aqueous solubility of otherwise poorly soluble compounds. Complexation with such compounds proceeds by a simple association reaction: CD + compound $\Leftarrow K_a \Rightarrow$ compound*CD (K_a: association constant; compound*CD: complex comprising compound and cyclodextrin). The association constant depends upon the type of CD used and the characteristics of the guest molecule, Accordingly, the complex in its free form will disassociate upon dilution. However, when the complex is encapsulated in liposomes, the CD concentration can be maintained at a high enough level to stabilize the complex. Therefore, stable CD*compound complexes can be maintained in liposomes at lower CD levels than would be the case for free CD*compound complexes. Chemically modified cyclodextrins (CMCDs) provide enhanced solubility in comparison to their parent cyclodextrins, and are therefore valuable for further enhancing the amount of poorly soluble compounds which may be incorporated into liposomes. CMCD complexes may be especially useful in connection with a lyophilized liposome preparation wherein crystallization need only be inhibited between the time of rehydration and administration.

The liposome may be administered to animals such that the hydrophobic molecule/bioactive agent is presented to the animals in a therapeutically useful form. The liposome may be administered alone, but will more commonly be given as part of a pharmaceutical composition comprising the liposome and a pharmaceutically acceptable carrier. For the purposes of this invention, a "pharmaceutically acceptable carrier" means any of the standard carriers, diluents, excipients and the like generally intended for use in connection with the administration of biologically active agents to animals. Such carriers are well known in the art and are generally chosen with regards to a number of factors, such as the particular drug being used and the intended route of administration, which are understood by the ordinarily skilled artisan.

Presently, it is preferred that the pharmaceutical compositions of this invention be administered intravenously. Accordingly, pharmaceutical carriers presently preferred for use in accordance with the practice of this invention are those well known carriers suitable for use in connection with intravenous administration of liposomes and include, but are not limited to, sterile aqueous solutions such as physiological saline, 5% dextrose USP solutions and various aqueous buffers, e.g., aqueous phosphate buffers. The total solute concentration in such carriers should be controlled to keep the composition

isotonic. Pharmaceutically acceptable carriers may also contain additional components, such as anti-oxidants, preservatives and the like, which are compatible with the active agent. The choice of such additional components is well within the purview of the ordinarily skilled artisan. Other carriers, e.g., tablets for oral administration and oils for mucosal or topical administration, may be prepared employing general knowledge and used in accordance with the practice of this invention.

Such pharmaceutical compositions may be administered to animals, e.g., mammals, and preferably humans, for the diagnosis, treatment or pevcention of diseases, disorders or conditions. Preferably, the bioactive agent is a therapeutic agent and the pharmaceutical composition is used in a method of treating an animal for a disease, disorder or condition which comprises administering to the animal a therapeutically effective amount of the pharmaceutical composition. "Therapeutically effective amount" as used herein means any amount of the pharmaceutical composition effective to treat, inhibit or prevent a disease, condition or disorder in an animal. Therapeutically effective amounts depend upon a number of factors within the purview of the ordinarily skilled artisan to determine including: the age, weight, size and general condition of the animal being treated; the type of disease being treated and the stage of its progression; and the type of liposome employed and the lipids used to prepare it. Typically, the therapeutically effective amount of the pharmaceutical composition is an amount comprising from about 1 milligram of the liposome of this invention per kg of the body weight of the animal to which the pharmaceutical composition is administered to about 1000 milligrams per kg, desirably from about 100 milligrams per kg of body weight to about 400 mg per kg. The ratio of bioactive agent to lipid in the liposome is typically from about 1:50 to about 1:5, but may be made higher or lower when it is necessary to do so.

In presently preferred embodiments of this invention the bioactive agent which is an integral component of the multicomponent bilayer is an anticancer agent. Accordingly, in presently preferred embodiments of this invention, the liposome provided is used in a pharmaceutical composition to treat an animal afflicted with a cancer. Cancers are treated, according to the practice presently preferred herein, with a liposome having a multicomponent bilayer comprising SCPC, DEPC, DMPC and taxol or camptothecin, but, most preferably, taxol. However, this invention is not limited to one specific embodiment of disease

treatment, or even to disease treatment. Rather, the multicomponent liposome may be employed for any diagnostic or therapeutic purpose for which a suitable bioactive agent may be found. For example, the liposome may be used to diagnose and/or treat diseases caused by bacterial, viral, fungal and parasitic infections or growth, aging or regenerative disorders.

Also provided herein is a unit dosage form of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the liposome of this invention. "Therapeutically effective amount" as used herein means any amount of the liposome effective to treat, inhibit or prevent a disease, condition or disorder in an animal. Such amounts will depend upon a number of factors within the purview of the ordinarily skilled artisan to determine and control including, but not limited to: the age, weight, size and general condition of the animal being treated; the type of disease, disorder or condition being treated; and the type of liposome employed and the lipids used to prepare it Typically, the therapeutically effective amount of the liposome is from about 1 mg of the multicomponent liposome per kg of the body weight of the animal to which the pharmaceutical composition is administered to about 1000 mg per kg of body weight. Desirably, the therapeutically effective amount is from about 100 mg per kg of body weight to about 400 mg per kg. Drug-to-lipid ratios in the liposome of this invention depend upon a number of factors, such as the relative proportions of the various lipid components, the nature of the bioactive agent and the type of liposome used, that are within the purview of the ordinarily skilled artisan to control. Typically, the drug-to-lipid ratio (w/w) is from about 1:50 to about 1:5, but may be higher or lower when necessary.

This invention will be better understood from the Examples which follow. However, those of ordinary skill in the art will readily understand that these examples are merely illustrative of the invention as defined in the claims which follow thereafter.

Examples

Example 1

Taxol/Lipid Association

Dispersion of taxol and a lipid were formulated, with the taxol and lipid concentrations (as well as the relative proportions of taxol and lipid) given in Table 1 (see below). The ability of taxol to form stable dispersions with each lipid is indicated (a "Yes" in Table 1 indicates that a stable dispersion was formed). The ability of taxol to form a dispersion with a particular lipid is an indicator of that lipid's potential suitability for formulation in a multicomponent bilayer.

TABLE 1

TAXOL ASSOCIATION WITH VARIOUS LIPIDS

Part A

Contents	Dispersion?	Taxol (mg/ml)	Lipid (mM)	Taxol: Lipid (µg/mg)	Lipid: Taxol (m/m)	Mole %
DMPC	Yes	0.5846	27.9	30.91	40.8	2.39
DPPC	Yes	0.4585	15.9	39.3	29.7	3.26
DSPC	Yes	0.6190	22.6	34.66	31.2	3.11
DMPG	Yes	0.7107	29.7	34.73	35.7	2.72
POPG	Yes	0.7222	27.1	34.31	32.0	3.03
SOPC	Yes	0.6878	28.7	30.41	35.6	2.73
POPC	Yes	0.7222	27.3	34.8	32.3	3
DOPC	Yes	0.7107	35.2	25.68	42.3	2.31
DEPC	Yes	0.6534	26.8	31.01	35.1	2.77
BPS: EPC (1:3, w/w)	Yes	0.8024	26.0	39.94	27.7	3.48
EPC	Yes	0.8024	24.0	43.99	25.6	3.76
CHS	Yes	0.7680	N/A	38.4	N/A	N/A
THS	No	N/A	N/A	N/A	N/A	N/A
MOPE	Micellar	0.182	N/A	9.1	N/A	N/A
DPhytPC	Yes	0.6763	23.7	33.7	29.90	3.24
DLPE	Poorly hydrated	0.5732	24.9	25.18	37.1	2.62
DOPE	No	N/A	N/A	N/A	N/A	N/A

Part B

	Crystals in 24 hours?	Crystals in 5 Days?	Crystals in 7 Months?
DMPC	Yes	N/A	N/A
DPPC	Yes	N/A	N/A
DSPC	Yes	N/A	N/A
DMPG	Yes	N/A	N/A
POPG	Yes	N/A	N/A
SOPC	No	No	Yes
POPC	No	No	Yes
DOPC	No	Yes (tubules)	N/A
DEPC	No	Yes	N/A
BPS:EPC 1:3 (w/w)	?	Yes	N/A
EPC	No	No	Yes
CHS	No	Yes	N/A
THS	N/A	N/A	N/A
MOPE	No	No	Yes
DPhytPC	No	No	Yes
DLPE	No	Yes	N/A
DOPE	N/A	N/A	N/A

The initial screening of lipid candidates was performed at a target taxol concentration of 30 micrograms taxol per milligram lipid, using a target lipid concentration of 20 milligrams per milliliter. The actual lipid concentration was determined for phospholipids by using a modified Bartlett assay. Taxol concentration was determined by UV absorbance, using the standard literature value of 29,800 liter mol^{-1} cm^{-1} at 228 nm for the molar extinction coefficient. In cases where lipid absorbed appreciably, a correction was made by subtracting the lipid contribution to the absorbance at that wavelength. Physical stability of the preparations were examined by light microscopy to determine the presence of taxol crystals, which appeared as needle-shaped aggregates.

Taxol crystals formed readily with gel phase saturated chain lipids; crystals also formed if the lipid headgroups had a negative charge or the acyl chains had transunsaturation. Taxol crystallization was observed in dispersions with most of the lipids tested within 24 hours to five days; crystallization was observed by seven months in all of the dispersions, indicating that taxol/lipid dispersions are of limited therapeutic utility.

Example 2

Taxol Solubility in EPC

The solubility limit of taxol in EPC was examined (see Table 2, below). The first study varied the taxol:lipid ratio at low lipid (20 mg/ml) concentrations as follows: multilamellar liposomes were prepared, in triplicate, at target concentrations ranging from 50 micrograms taxol per milligram lipid to 300 micrograms taxol per milligram lipid. The target concentration of lipid was 20 milligrams per milliliter.

TABLE 2

SOLUBILITY OF TAXOL IN EPC

Taxol:Lipid Ratio (MG TAXOL/MG EPC)	Crystals After 48 Hours?	Crystals After 5 Days?
50	No	Yes
50	No	Yes
50	No	No
100	Yes	Yes
100	Yes	Yes
100	Yes	Yes
150	Yes	Yes
150	Yes	Yes
150	Yes	Yes
200	Yes	Yes
200	Yes	Yes
200	Yes	Yes
250	Yes	Yes
250	Yes	Yes
250	Yes	Yes
300	Yes	Yes
300	Yes	Yes
300	Yes	Yes

Within 5 days, crystals were visible in all samples except one at the lowest taxol/lipid ratio. The rate of crystal formation is rapidly accelerated at higher taxol:lipid ratios. A subsequent investigation examined the stability of EPC taxol multilamellar vesicles as a function of taxol:lipid ratios at a higher target lipid concentration (100 mg/ml). The target taxol:lipid ratios were 20, 30,

40, 50, 60, 70, and 80 micrograms taxol per milligram lipid. Within one week, crystals were evident in all samples except at the lowest taxol:lipid ratio.

Example 3

Differential Scanning Calorimetry for DEPC/DMPC/SCPC Liposomes

Differential scanning calorimetry (DSC) was conducted for a sample of a DEPC/DMPC/SCPC mixture (4 mg/ml lipid) with no taxol present. The nominal scan rate was 20 degrees per hour. Results from the DSC (see Figure 3, top trace) are consistent with the absence of any large scale phase separation in this ternary (three component) composition

In order to determine whether the DEPC/DMPC/SCPC composition at the chosen proportions (.375 DEPC: .325 DMPC: .300 SCPC) is at the optimal point on the three component phase diagram, combinations of these three lipids in other than optimal proportions were also prepared and examined by DSC (see Table 3, below).

TABLE 3

DEPC/DMPC/SCPC TERNARY COMPOSITIONS
(Mole Fraction)

Sample	DEPC	DMPC	C(18):C(10)PC
a	0.525	0.175	0.30
b	0.525	0.325	0.15
c	0.375	0.475	0.15
d	0.225	0.475	0.30
e	0.225	0.325	0.45
f	0.375	0.175	0.45

Figures 4A-4G are a three dimensional graphical representation of a parameter derived from these DSC studies. The base of the cube contains the triangular three component phase diagram, plotted in the x-y plane. The vertical (z) axis can represent any single experimental measurement; for these figures, the transition onset temperature is plotted. A three dimensional surface can be drawn through these data. A minimum in the onset temperature corresponds with the chosen ternary composition (0.375 DEPC: 0.325 DMPC:

0.300 SCPC), indicating that with respect to the resolution of this experiment, the composition initially chosen is near an optimal point.

Example 4

X-ray Diffraction Analysis

Low angle x-ray diffraction of this DEPC/DMPC/SCPC ternary composition was examined using multilamellar liposomes, in order to compare the x-ray signatures in systems with, and without, taxol incorporated.

The data (see Table 4, below) indicates that a single lamellar repeat distance occurs when the ternary system has taxol incorporated, whereas in the absence of taxol, two distinct lamellar repeat distances are noted. In the absence of taxol, the lipids within the bilayer likely rearrange acyl chain packing in order to fill the voids which exist because of the acyl chain asymmetry. However, the domain size must be on the order of molecular dimensions, since large scale lateral phase separation is not observed by DSC. When taxol is present, a single continuous phase results, with a single characteristic lamellar repeat distance below and above the phase transition temperature, indicating that the end-to-end packing of the asymmetric lipid, as shown in Figure 1, is likely to occur.

TABLE 4

SMALL ANGLE X-RAY SCATTERING

Sample Temp.	With Taxol	Without Taxol
2 dg. C	66.6[1]	66.5 and 73.4
20 dg. C	75.1	60.5 and 65.6

[1] Bilayer repeat spacing in ternary lipid system (A°)

Example 5

Differential Scanning Calorimetry Analysis

In order to more fully characterize the DEPC/DMPC/SCPC ternary composition as a function of added taxol, samples were prepared at target taxol:lipid ratios of 0, 10, 20, 30, 40, 50, 60, 70, 80 micrograms taxol per milligram lipid. These samples were characterized by differential scanning calorimetry (see Figure 3).

While the addition of taxol broadens the transition and lowers the T_m slightly, a large fraction of the transition enthalpy persists, even at high taxol:lipid ratios, indicating that the incorporation of taxol does not disrupt the acyl chain packing to the extent that the population of gauche conformers increases dramatically. If taxol were to perturb the chain packing and introduce increasing amounts of gauche conformers in the hydrocarbon core of the lipid bilayer, the transition enthalpy could be expected to gradually decrease to zero.

Example 6

Incorporation of Other Drug Molecules Into DEPC/DMPC/SCPC Liposomes

Molecules of pharmaceutical interest other than taxol (see Figure 3A) were examined for their ability to be an integral component of a multicomponent bilayer. Nogalamycin (antitumor) (Fig 3B), Janssen R51211 [itraconazole] (antifungal) (Fig 3C); camptothecin (antitumor) (Fig 3D); and pregnenolone (steroid) (Fig 3E) were incorporated into a DEPC/DMPC/SCPC multicomponent bilayer by adding the compounds to a mixture of the lipids (at mole ratios of 0.375, 0.325 and 0.300 for DEPC, DMPC and SCPC, respectively) prior to their formation into liposomes. The compounds were prepared at approximately 3 mole percent with respect to lipid, which corresponds roughly with one molecule per bilayer pocket. All of the compounds share the common trait of vanishingly low water solubility.

Only itraconazole appeared to form crystals. An examination of its structure reveals that an itraconazole molecule is a long, linear rod shaped molecule, whose longest dimension could conceivably exceed the size of the

hydrophobic pocket, thus leading to a poor fit. Increasing the size of the pocket should result in a successful incorporation.

Example 7

Single Dose Toxicity Studies

As discussed hereinabove, taxol is currently administered as a cremophor-based suspension. Typically, this suspension is given at 250 mg/sqm, intravenously over a 24-hour period. It is accompanied by administration of dexamethasone (20 mg, at 7 and 14 hours prior to the administration of taxol), diphenyhdramine and cimetidine (25 mg and 300 mg, respectively, intravenously 1 hour prior to taxol administration), and diphenydramine and epinephrine (on an "as needed" basis during infusion).

However, subjects exhibit myelosuppression, primarily leukopenia, and neurotoxicity when the taxol dose exceeds a certain level. Such toxicity is dose-dependent and reversible, and occurs with an incidence of about 1/infusion rate. Other drug related toxicities observed to occur in subjects given taxol include mucositis, nausea and vomiting, alopecia and fever. The subjects also exhibit vehicle-related toxicities such as anaphylactoid reaction, rashes, pruritus and flushing. Such toxicities are observed to occur at a rate about equal to the infusion rate.

Single dose toxicity studies comparing non-liposomal taxol, egg PC multilamellar liposomes containing taxol and DEPC/DMPC/SCPC multilamellar liposomes containing taxol were performed in a mouse model system. The dose of taxol lethal to 10% (LD10), 50% (LD50) and 90% (LD90) of the sample population were measured for taxol given as a dispersion in a polysorbate base, in a Cremophor suspension, in EPC multilamellar vesicles and in the liposomes (ternary lipid multicomponent bilayer) of this invention. The results of these studies are set forth in Table 5 (see below).

TABLE 5

SINGLE DOSE LETHALITY IN CDF1 MICE
ADMINISTERED TAXOL BY THE INTRAPERITONEAL ROUTE

Taxol Vehicle	Dose (mg/kg)			Predictor Equation[1]	
	LD10	LD50	LD90	m	b
Polysorbate	24.9	68.8	189.7	2.91	-0.34
Cremophor	8.0	16.2	32.6	4.21	-0.09
EPC	24.9	68.6	189.7	2.91	-0.34
Ternary Lipid[2]	24.9	68.6	189.7	2.91	-0.34

[1] % Dead = log dose + b; "m," is the slope of a graphical representation of the predictor equation and "b," its y intercept. [2] DEPC/DMPC/SCPC liposomes.

These data show that taxol in a polysorbate base, EPC liposomes and the liposomes of this invention exhibited less toxicity than when administered as a cremophor-based suspension. For instance, the LD50, that dose toxic to 50% of the sample population, was 16.2 mg per kg for cremophor-based taxol, and over four times higher for the other formulations.

Example 8

Efficacy Studies

Efficacy studies comparing non-liposomal taxol, egg PC multilamellar liposomes containing taxol and DEPC/DMPC/SCPC multilamellar liposomes containing taxol were performed in a B16 melanoma model in mice, with (ip) cell administration and (ip) dosing.

BDF1 (C57BL6 x DBA/2) female mice weighing 14-20 grams at the onset of treatment were used. B16 melanoma cells (0.5 ml of 10% Brei) were injected into these mice intraperitoneally at day zero. Administration of taxol was intraperitoneally at 1, 5 and 9 days after injection of the B16 cells. Taxol was formulated into EPC liposomes, by adding it to the EPC prior to liposome formation. Taxol was formulated into DEPC/DMPC/SCPC multilamellar liposomes by adding it to a mixture of these lipids (at mole ratios of 0.375, 0.325

and 0.300 for DEPC, DMPC and SCPC, respectively) prior to liposome formation. The drug was administered as a dispersion with polysorbate base, in a Cremophor suspension, in EPC multilamellar vesicles and in DEPC/DMPC/SCPC multilamellar liposomes at the indicated dosages (6.25, 12.5, 25, 50 and 100 mg per kg of body weight per day of taxol, and 18.75, 37.5, 75, 150 and 300 mg per kg total taxol). Also administered were "vehicle controls" of polysorbate base or cremophor base alone, i.e., with no taxol associated. The animals were weighed at the first, fifth, ninth and thirteenth days of the treatment period, and were sacrificed on the sixtieth day. The median day of death seen in the sample populations, the %T/C ((100 x median day of death of treated animals)/(median day of death of untreated animals)), the number of long term survivors and the occurrence of toxic deaths was measured. Tables 7 and 8 (see below) set forth the results of the studies. Table 6 sets forth data demonstrating the antitumor activities of taxol in a B16 mouse melanoma that was obtained from publicly available information from the National Cancer Institute (NCI).

TABLE 6

ANTITUMOR ACTIVITIES OF TAXOL IN B16 MELANOMA (NCI)

Tumor System	Route tumor/drug	Dosage	Dose Range (mg/kg/inj.)	OD	%T/C at OD	Eval.[1]
B16	IP/IP	Daily x 9	1.25-80	5	283	++
B16	IP/IP	q4d x 3	6.25-100	25	254	++
B16	IP/IP	q8h x 2 (days 1-9)	0.66-11.2	5	240	++
B16	IP/PO	Daily x 9	1.25-40	20	108	-
B16	IP/IV	Daily x 5	0.66-11.2	11.2	108	-
B16	SC/IP	Daily x 9	1.3-22.5	4.4	126	-

[1]: Evaluation of antitumor activity = ++: highly active; +: active; -: inactive. PO = peroral. OD = optimal dose.

TABLE 7

EFFECT OF TAXOL/TREATMENT METHOD IN ASCITIC B16 MELANOMA MODEL

Treatment	Dose (mg/kg) Daily	Dose (mg/kg) Cumulative	Median Day of Death	%T/C	Long Term Survivor	Toxic Death
None	N/A	N/A	19.0	N/A	0	N/A
Vehicle Control						
Polysorbate Base	1		20.5	108	0	---
Cremophor Base	1		19.0	100	0	---
Polysorbate Base						
	6.25	18.75	29.0	153	0	---
	12.5	37.5	27.5	145	0	---
	25	75	26.0	137	1	---
	50	150	15.0	79	0	Yes
	100	300	7.5	39	0	Yes
Cremophor Suspension						
	6.25	18.75	25.0	132	1	---
	12.5	37.5	28.0	147	0	---
	25	75	30.0	158	1	---
EPC Liposomes						
	6.25	18.75	27.5	145	0	---
	12.5	37.5	32.5	171	0	---
	25	75	38.5	203	0	---
	50	150	35.0	184	0	---
	100	300	10.5	55	0	Yes
DEPC/DMPC/SCPC Liposomes						
	6.25	18.75	26.0	137	1	---
	12.5	37.5	33.0	174	2	---
	25	75	41.0	216	1	---
	50	150	44.0	232	1	---
	100	300	15.0	79	0	Yes

[1] Equivalent volume to highest taxol dose. % T/C = (100 x median day of death of treated animals)/(median day of death of untreated animals) [excludes survivors]. Long term survivors: tumor-free animals surviving to the end of the experimental period.

TABLE 8

EFFECT OF TAXOL IN THE ASCITIC B16 MELANOMA MODEL

Dosage Form	OD (mg/kg/ day)	% T/C	# of Long Term Survivors
Polysorbate base	25	137	1/10
Cremophor base	25	158	1/10
EPC liposomes	25	203	0/10
Ternary system	50	232	1/10

The data indicate that for non-liposomal dosage forms of taxol, the percent T/C at the optimal dose was lower than expected based upon literature values (which indicate that with an optimal dose of 25 mg of taxol per kg of body weight per day, the percent T/C equals 240). These non-liposomal formulations of taxol were unstable, which may have contributed to the variation observed. For liposomal taxol, potency (effect/unit mass) compared favorably to the non-liposomal formulations.

Example 9

Efficacy Studies

Efficacy studies comparing non-liposomal taxol, egg PC multilamellar liposomes containing taxol and ternary lipid multilamellar liposomes containing taxol were performed for a P388 leukemia model in mice, with (ip) cell administration and (ip) dosing.

BDF1 (C57BL/6 x DBA/2) female mice weighing 14-20 grams were used in this study. These mice were injected with P388 leukemia cells (1×10^6), intraperitoneally, at day zero. Taxol was administered to the animals, intraperitoneally, at the first, fifth and ninth days of the treatment period at the dosages indicated in Table 10 (see below). The animals were sacrificed on the sixtieth day. The median day of death in the sample population, the % T/C, the number of long term survivors and the occurrence of toxic deaths were determined (see Table 10). Table 11 (see below) shows the %T/C exhibited when mice were administered P388 leukemia cells and the optimal daily dose of taxol,

either as a dispersion with polysorbate, in a Cremophor suspension, formulated in EPC liposomes or formulated in DEPC/DMPC/SCPC liposomes. Table 9 (see below) presents data derived from publicly available sources indicating the known antitumor activities of taxol in the indicated tumor systems.

TABLE 9

ANTITUMOR ACTIVITIES OF TAXOL IN MURINE LEUKEMIAS

Tumor System	Route tumor/drug	Regimen	Dose Range (mg/kg/inj)	Opt. Dose	%T/C at OD	Eval.
L1210	ip/ip	dailyx15	10-35	20	131	+
P388	ip/ip	single	2.6-9	9	115	-
P388	ip/ip	dailyx5	2.5-20	20	157	+
P388	ip/ip	dailyx9	1.25-40	5	149	+
P388	ip/ip	q4dx3	25-120	43	170	+
P388	ip/sc	dailyx9	1.25-80	40	125	+
P388	ip/po	dailyx9	2.5-160	40	104	-
P1434	ip/ip	dailyx10	1.88-15	3.75	300	++

Eval.: ++ = highly active against tumor; + = active; - = inactive.

TABLE 10

EFFECTS OF TAXOL IN P388 MURINE LEUKEMIA MODEL

Treatment	Dose (mg/kg) Daily	Cum.	Median Day of Death	% T/C	Long Term Survival	Toxic Death
None	N/A	N/A	11.0	N/A	0	N/A
Vehicle Control						
Polysorbate		1	10.0	91	0	NO
Cremophor		1	11.5	105	0	NO
Polysorbate Base						
	6.25	18.75	13.0	118	0	NO
	12.5	37.5	13.0	118	0	NO
	25	75	12.5	114	0	NO
	50	150	14.0	127	0	NO
	100	300	10.0	91	0	NO
Cremophor Base						
	6.25	18.75	11.5	105	0	NO
	12.5	37.5	12.0	109	0	NO
	25	75	15.5	141	0	NO
	50	150	10.5	95	0	NO
EPC Liposomes						
	6.25	18.75	14.0	127	0	NO
	12.5	37.5	14.5	132	0	NO
	25	75	15.0	136	0	NO
	50	150	17.5	159	0	NO
	100	300	12.0	109	0	NO
DEPC/DMPC/SCPC Liposomes						
	6.25	18.75	13.0	118	0	NO
	12.5	37.5	14.5	132	0	NO
	25	75	15.0	136	0	NO
	50	150	19.5	177	0	NO
	100	300	14.5	132	0	NO

[1] Equivalent volume to highest taxol dose. % T/C = (100 x median day of death of treated animals)/(median day of death of untreated animals) [excludes survivors]. Long term survivors: tumor-free animals surviving to the end of the experiment.

TABLE 11

P388 MURINE LEUKEMIA MODEL RESULTS

Dosage Form	Optimal Dose (mg per kg per day)	% T/C
Polysorbate Base	50	127
Cremophor Base	25	141
EPC Liposomes	50	159
Ternary System	50	177

The data show that the DEPC/DMPC/SCPC taxol formulations of this invention are more effective at reducing the effects of P388 leukemia cells in mice than are presently available formulations of taxol. For all taxol doses greater than 6.5 mg/kg/day, the median day of death was higher for animals administered the taxol-containing liposomes of this invention than for animals treated with taxol/cremophor. For all doses tested, %T/C, a measure of the relative survival of treated and untreated populations, was higher for animals given DEPC/DMPC/DSPC liposomes containing taxol. The data show that potency (effect/unit mass) of the liposomal forms were comparable to the non-liposomal forms. The data suggest that the liposomal forms may be dosed to a higher level than the non-liposomal drug.

Example 10

Efficacy Studies

Efficacy studies comparing non-liposomal taxol with DEPC/DMPC/SCPC multilamellar and LUVET liposomes containing taxol have been performed in mice using B16 melanoma cells, with (sc) cell administration and (iv) drug dosing. Both an early stage implanted tumor model and a late stage implanted tumor model were used. BDF1 (C57BL/6 x DBA/2) female mice weighing 14-20 grams were used in this study. These mice were injected with B16 cells (0.5 ml of 10% Brei), subcutaneously, at day zero. Taxol was administered to the animals, intravenously, at the first through fifth days after injection of the cells at the doses indicated in Tables 12 and 13 (see below). %T/C, the number of long term survivors and tumor growth inhibition were measured. Control

animals given melanoma cells, but no taxol, were also examined. The results are reported in Tables 12 and 13.

TABLE 12

RESULTS WITH TAXOL ADMINISTRATION IN THE EARLY B16 MELANOMA MODEL

Dosage Form	Dose (mg/kg) Daily	Cum.	%T/C	Survival Time Long Term Survivors	Tumor Growth Inhibition
Control					
	N/A	N/A	N/A	0	N/A
Cremophor Base					
	3.13	15.65	115	0	120
	6.25	31.25	106	0	157
	12.50	62.50	132	3	60
	25.00	125.0	136	0	49
DEPC/DMPC/SCPC MLVs					
	3.13	15.65	109	0	84
	6.25	31.25	155	0	78
	12.5	52.50	140	2	38
	25.00	62.50	30	0	--
DEPC/DMPC/SCPC LUVETs					
	3.13	15.65	130	1	71
	6.25	31.25	157	0	60
	12.50	62.50	151	0	49
	25.00	125.0	157	1	29
	50.00	250.0	28	0	--

Tumor growth inhibition = (100 x median tumor weight of treated on day = t)/(median tumor weight of control on day = t).

TABLE 13

RESULTS OF THE ADMINISTRATION OF TAXOL IN LATE B16 MELANOMA MODEL

	Dose (mg/kg)			Survival Time	Tumor Regression	
Dosage form	Daily	Cum.	% T/C	Long Term Survivors	Part.	Complete
Control	N/A	N/A	N/A	0	0	0
Cremophor Base						
	3.13	15.65	96	0	0	0
	6.25	31.25	90	0	0	0
	12.5	62.50	92	0	0	0
	25.00	125.0	122	0	0	0
DEPC/DMPC/SCPC MLVs						
	3.13	15.65	90	0	0	0
	6.25	31.25	100	0	1	0
	12.5	62.50	112	0	0	0
	25.00	125.0	88	0	2	0
	50.00	250.0	67	---	---	---
DEPC/DMPC/SCPC LUVETs						
	3.13	15.65	104	0	0	0
	6.25	31.25	92	0	0	0
	12.5	62.50	112	0	3	0
	25.00	125.0	131	0	3	0
	50.00	250.0	90	0	6	0

Regressions: partial (part.): greater than or equal to a 50% reduction in tumor size; complete: reduction to below palpable limits.

The data demonstrate that the liposome of this invention was a safe and effective delivery vehicle for taxol. The DEPC/DMPC/SCPC liposomes containing taxol were better able to increase the proportion of survivors amongst treated animals and were also better able to inhibit tumor growth.

Example 11

Stability Studies

Stability studies involved formation of the various preparations indicated below; and the preparations were monitored for the formation of visible crystals using phase-contrast light microscopy at 400x magnification. The target concentrations of drug and lipid in each set of experiments are indicated below.

(A) TAXOL CRYSTAL FORMATION IN EPC MLV's:

The following samples were prepared at a lipid concentration of 20 mg/ml: 50 micrograms taxol/mg lipid (A); 100 micrograms taxol/mg lipid (B); 150 micrograms taxol/mg lipid (C); 200 micrograms taxol/mg lipid (D); 250 micrograms taxol/mg lipid (E). Samples B - E had visible crystals two days after sample hydration. Two out of three sample A's had crystals present 5 days after sample hydration. All samples had crystals present 2 weeks after hydration.

The following samples were prepared at a lipid concentration of 100 mg/ml: 20 micrograms taxol/mg lipid (A); 30 micrograms taxol/mg lipid (B); 40 micrograms taxol/mg lipid (C); 50 micrograms taxol/mg lipid (D); 60 micrograms taxol/mg lipid (E); 70 micrograms taxol/mg lipid (F); and 80 micrograms taxol/mg lipid (G). On the first day after hydrating the samples, no visible crystals were present in any of these samples. On the second day after hydrating the samples, samples E, F and G had visible crystals. On the sixth day after hydrating the samples, all except sample A had visible crystals.

The following sample was prepared at a lipid concentration of 20 mg/ml: 30 micrograms taxol/mg lipid. No crystals were visible 1, 5, and 30 days after sample hydration. Crystals developed in the sample between day 30 and day 100.

The following samples were all EPC/Taxol MLV's prepared for animal studies. All samples developed crystals within one week of preparation: A) 200 mg/ml lipid; 25 microgram taxol/mg lipid; B) 200 mg/ml lipid; 20 microgram taxol/mg lipid; C) 200 mg/ml lipid; 20 micrograms taxol/mg lipid.

(B) TAXOL CRYSTAL FORMATION IN DEPC/DMPC/SCPC LUVETs

The following sample was at a concentration of 187 mg/ml lipid; 21.3 micrograms taxol/mg lipid: This sample was extruded to form 100-nm LUVETs. No crystals are visible after 150 days; some vesicle fusion to form larger structures was noted.

The following sample was at a lipid concentration of 200 mg/ml; 25.0 micrograms taxol/mg lipid. Crystals were visible after 160 days.

The following sample was at a lipid concentration of 187 mg/ml; 21.3 micrograms taxol/mg lipid. No crystals were visible at 150 days.

The following sample was at a lipid concentration of 200 mg/ml; 20.0 micrograms taxol/mg lipid. No crystals were visible at 120 days.

The following samples were all 20 mg/ml lipid: 37.5 microgram taxol/mg lipid (A); 50 microgram taxol/mg lipid (B); and 62.5 microgram taxol/mg lipid (C). Sample C had visible crystals within two weeks; samples A and B did not exhibit taxol crystal formation by the 170th day of observation.

It appears that the stability of taxol in EPC liposomes is determined by a combination of taxol:lipid ratio as well as the actual taxol (and lipid) concentrations. At high taxol and lipid concentrations, the taxol:lipid ratio corresponding to a stable preparation is correspondingly less than the taxol:lipid ratio achievable at low taxol and lipid concentrations. In other words, an infinitely dilute preparation of taxol:EPC will be able to achieve the highest taxol:lipid ratio. This is in contrast to the liposomes of this invention, where the stability is only a function of the taxol:lipid ratio. Therefore, in these liposomes, a high taxol:lipid ratio may be achieved even in a concentrated preparation.

Example 12

Method of Preparing Ternary Lipid Multicomponent Liposomes Containing Taxol

SCPC, DEPC and DMPC were combined with taxol in a suitable solvent, e.g., tert-butanol, at a concentration of 40 mg lipid/taxol per ml solvent. The resulting mixture was lyophilized, after which the dried film was rehydrated

with buffer to form multilamellar vesicles. LUVETs were formed from these MLVs by extrusion through filters according to the procedures described in Cullis et al. (U.S. Patent No. 5,008,050) and Loughrey et al. (U.S. Patent No. 5,059,421).

Preparation of 1 ml of SCPC, DEPC, DMPC/taxol liposomes, at 200 mg/ml lipid and 40 micrograms taxol per mg lipid requires: 82.06 mg DEPC; 61.33 mg DMPC; 56.61 mg SCPC; and 8 mg taxol. These ingredients are combined in 5.2 ml t-butanol, following which, the mixture is lyophilized. The resulting film is rehydrated with about 0.8 ml buffer to reach the final 200 mg/ml lipid concentration.

Example 13

Antitumor Effect of Taxol Liposomes and Cremophor Taxol Against Established Mouse Colon 26 Carcinoma (C26)

Thirty female Balb/c mice weighing 20-22 grams each were injected subcutaneously with 1 x 10^6 C26 cells at day 0. The mice were randomly separated into control, liposomal taxol and cremophor taxol groups, each containing 10 mice. The groups treated with a taxol formulation were administered intravenously, at days 8, 9, 15, 16, 21 and 22, a taxol dose of 25 mg per kg of body weight each day. Weight and tumor size in each mouse were measured twice weekly until the tumors reaches 2 cm^3. Survival was assessed at day 120. The tumor volumes and survival in the groups were then compared. Results of these studies are presented in Table 14 (see below).

The data show that the ternary lipid multicomponent liposomal taxol formulations inhibited tumor growth to a greater extent than did the cremophor-based taxol formulations. The data also shows that the cremophor formulations were toxic at the same dose level.

TABLE 14

EFFECT OF TAXOL ON THE GROWTH OF ESTABLISHED C26 (S.C.) COLON CARCINOMA

Dose Form	**Dose (mg/kg)**	**GROWTH INHIBITION[a]** 12	20	29	**% T/C**	**Long Term Survivors[b]**
Buffer	-----	-----	-----	-----	100	0/10
Cremophor	25	47	27	1	55	0/10
Ternary	25	64	45	19	110	0/10

[a] Growth inhibition = percent reduction in tumor size in comparison to controls, on stated days; [b] number of long term survivors (120 days) out of total number of mice treated (10) in each group.

What is claimed is:

1. A liposome having a multicomponent bilayer comprising:

(a) a first amphipathic lipid which comprises an acyl chain of length A and an acyl chain of length B, wherein A and B are integers equal to the numbers of carbon atoms in the acyl chains and A is greater than B;

(b) a second amphipathic lipid which comprises an acyl chain of length C and an acyl chain of length D, wherein C and D are integers equal to the numbers of carbon atoms in the acyl chains and C is greater than or equal to D; and

(c) a bioactive agent,

wherein the difference between A and B is greater than or equal to the difference between C and D and wherein the bioactive agent is an integral component of the bilayer.

2. The liposome of claim 1, wherein A equals C.

3. The liposome of claim 1, wherein C equals D.

4. The liposome of claim 1, wherein the difference between A and B is greater than the difference between C and D.

5. The liposome of claim 1, wherein the difference between A and B is equal to the difference between C and D.

6. The liposome of claim 1, wherein the first and second amphipathic lipids are phospholipids.

7. The liposome of claim 6, wherein the phospholipids are phosphatidylcholines.

8. The liposome of claim 1, wherein the first and second amphipathic lipids comprise a bipolar lipid.

9. The liposome of claim 1, wherein the bioactive agent is a therapeutic agent.

10. The liposome of claim 9, wherein the therapeutic agent is an anticancer, antifungal, antibacterial, antiviral, antiparasitic, anti-aging, anti-inflammatory or growth-promoting agent.

11. The liposome of claim 10, wherein the therapeutic agent is an anti-cancer agent.

12. The liposome of claim 11, wherein the anti-cancer agent is taxol, camptothecin or nogalamycin.

13. The liposome of claim 1, wherein the multicomponent bilayer further comprises a phase separation prevention component comprising an amphipathic lipid.

14. The liposome of claim 1, wherein the first amphipathic lipid is C(18):C(10)-PC.

15. The liposome of claim 14, wherein the second amphipathic lipid is C(18:1):C(18:1)-PC and the multicomponent bilayer further comprises a phase separation prevention component comprising an amphipathic lipid.

16. The liposome of claim 15, wherein the phase separation prevention component comprises C(14):C(14)-PC.

17. The liposome of claim 15, wherein the bioactive agent is an anti-cancer agent.

18. The liposome of claim 17, wherein the anticancer agent is taxol.

19. The liposome of claim 17, wherein the anticancer agent is camptothecin.

20. The liposome of claim 1, further comprising a second bioactive agent.

21. The liposome of claim 1, further comprising an amount of a cyclodextrin effective to inhibit crystallization of the bioactive agent.

22. The liposome of claim 1, wherein the liposome has been dehydrated.

23. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the liposome of claim 1.

24. A method of treating an animal afflicted with a disease which comprises administering to the animal a therapeutically effective amount of the pharmaceutical composition of claim 23.

25. The method of claim 24 wherein the animal is a mammal.

26. The method of claim 25 wherein the mammal is a human.

27. The method of claim 24, wherein the disease is a cancer.

28. The method of claim 27, wherein the cancer is brain, ovarian, lung, colon or breast cancer.

29. The method of claim 27, wherein the disease is a cancer and the liposome comprises a multicomponent bilayer comprising C(18):C(10)-PC, C(18:1):C(18:1)-PC, (C(14):C(14)-PC) and taxol.

30. The method of claim 27, wherein the disease is a cancer and the liposome comprises a multicomponent bilayer comprising C(18):C(10)-PC, C(18:1):C(18:1)-PC, (C(14):C(14)-PC) and campthothecin.

31 The method of claim 24, wherein the disease is caused by a bacterial, viral, fungal or parasitic infection.

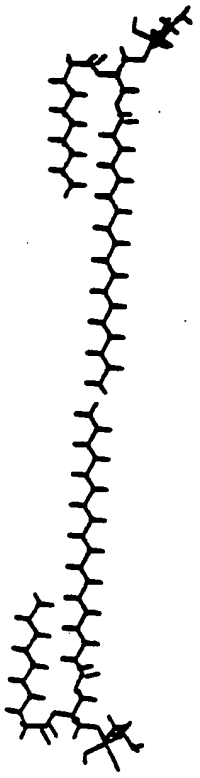
32. The method of claim 24, wherein the disease is a steroidal, regenerative or growth-associated disorder.

33. A unit dosage form of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of the liposome of claim 1.

34. The unit dosage form of claim 33, wherein the therapeutically effective amount is from about 1 milligram of the liposome per kg of the body weight of the animal to which the pharmaceutical composition is administered to about 1000 milligrams per kg.

35. The unit dosage form of claim 34, wherein the therapeutically effective amount is from about 100 milligrams per kg of body weight to about 400 mg per kg.

FIGURE 1



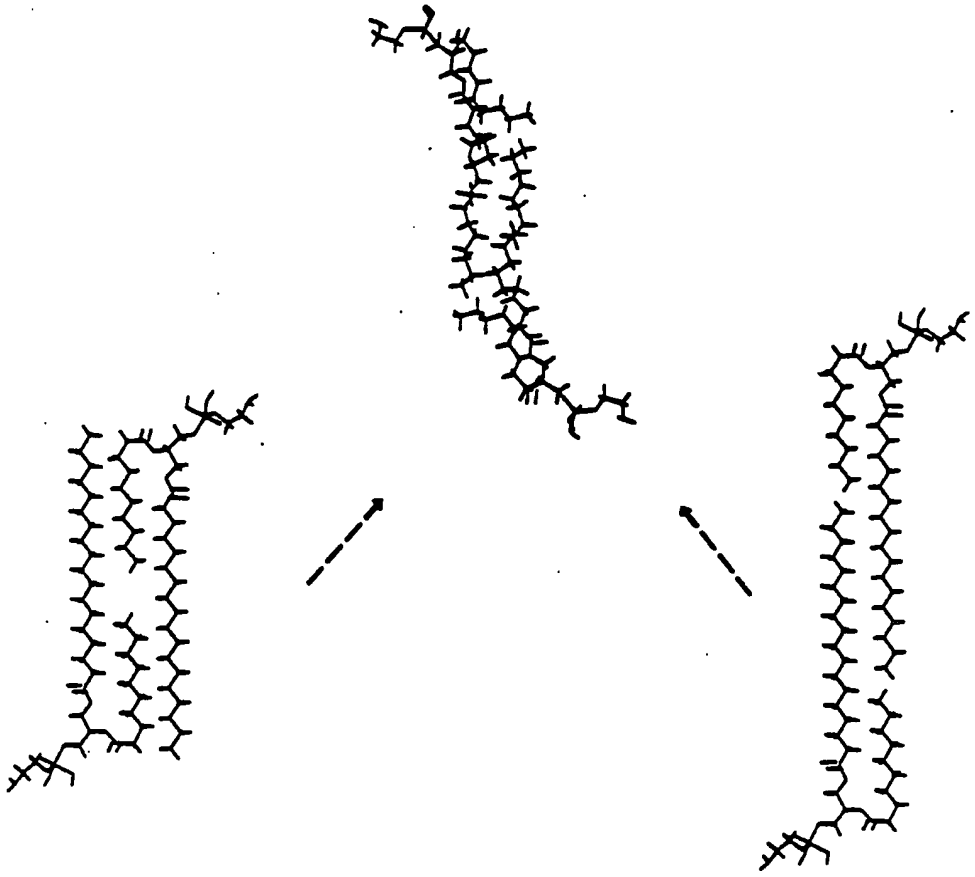


Fig. 2

Fig. 3a

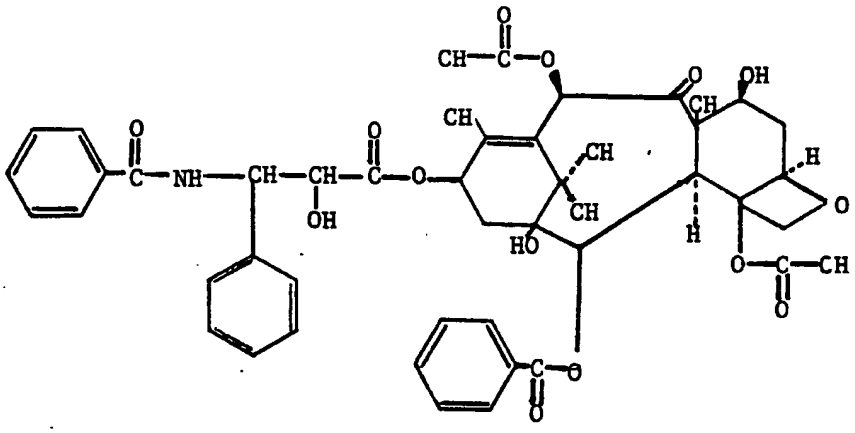


Fig. 3b

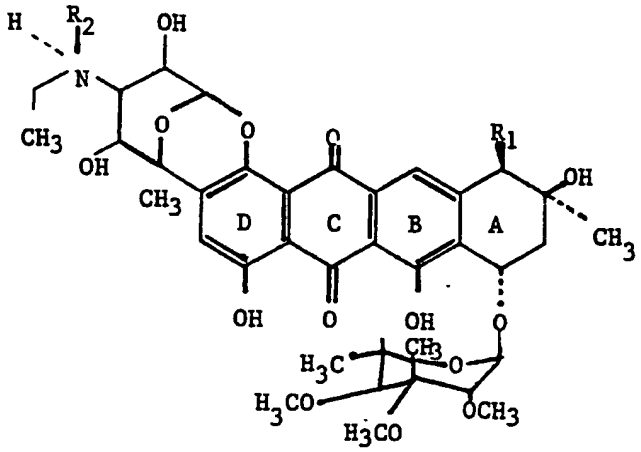


Fig. 3c

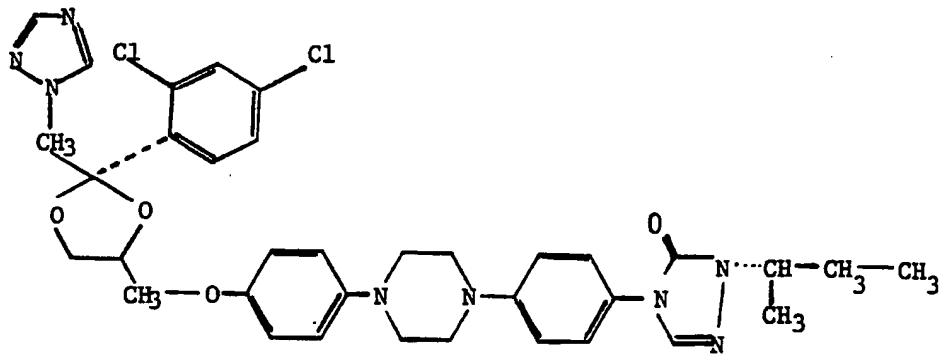


Fig. 3d

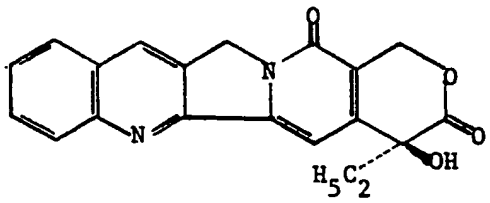
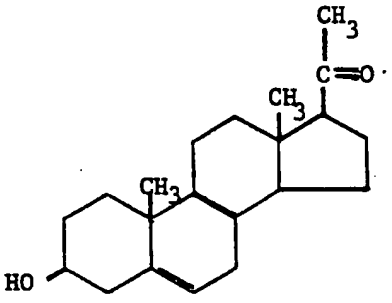


Fig. 3e



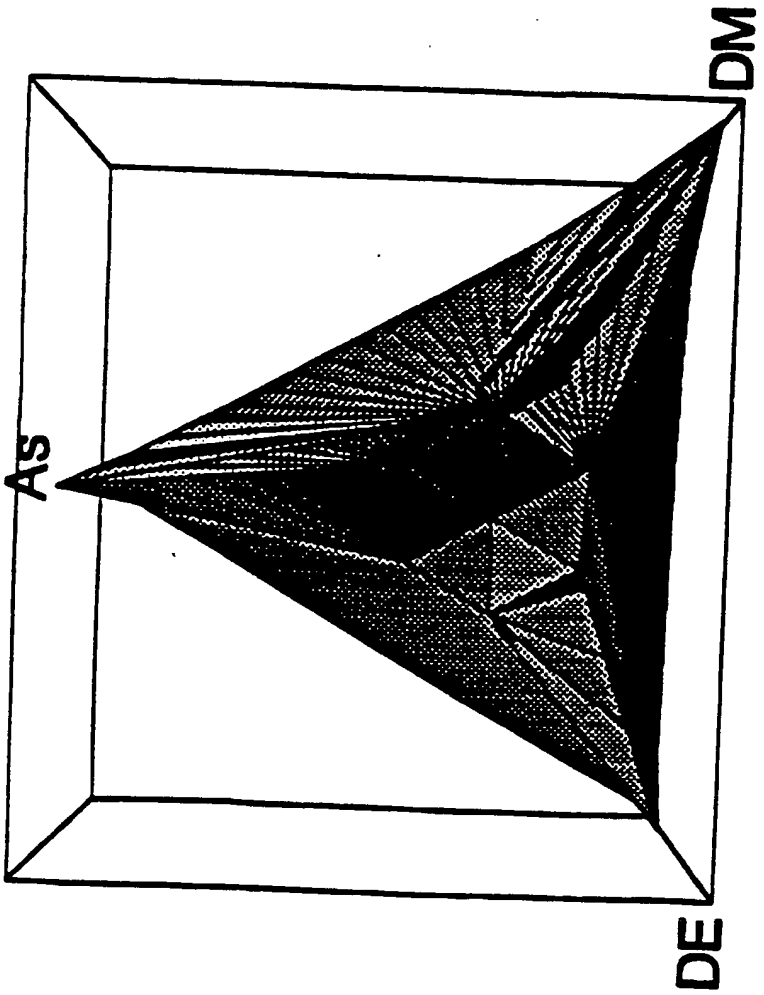


FIGURE 4A

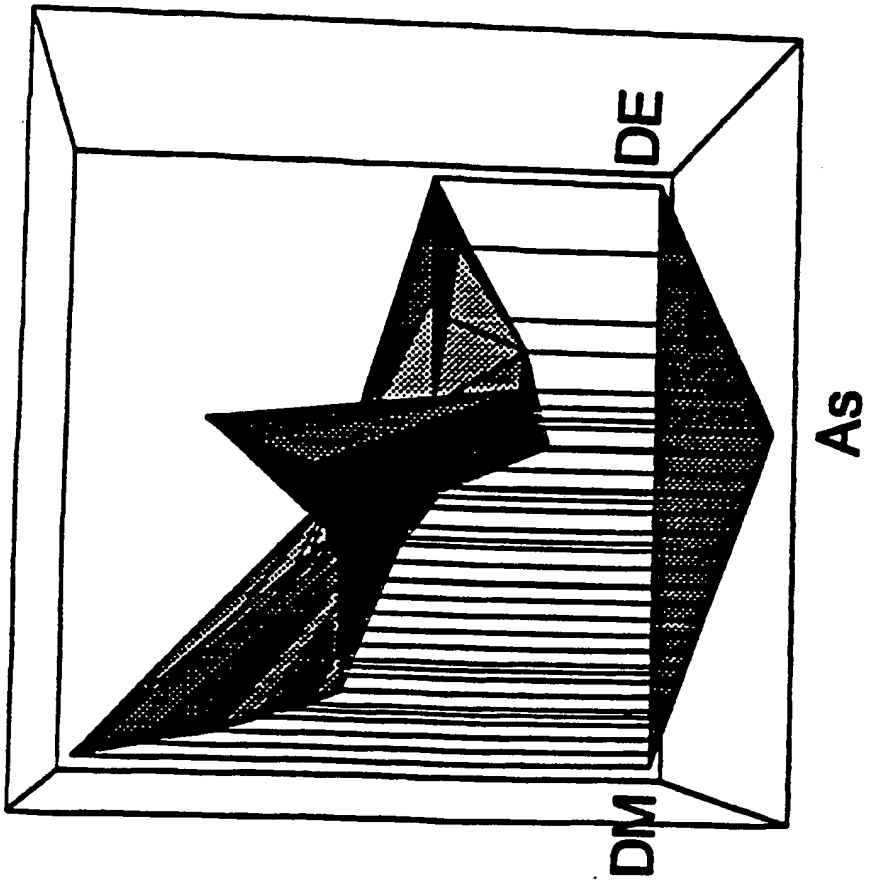


FIGURE 4B

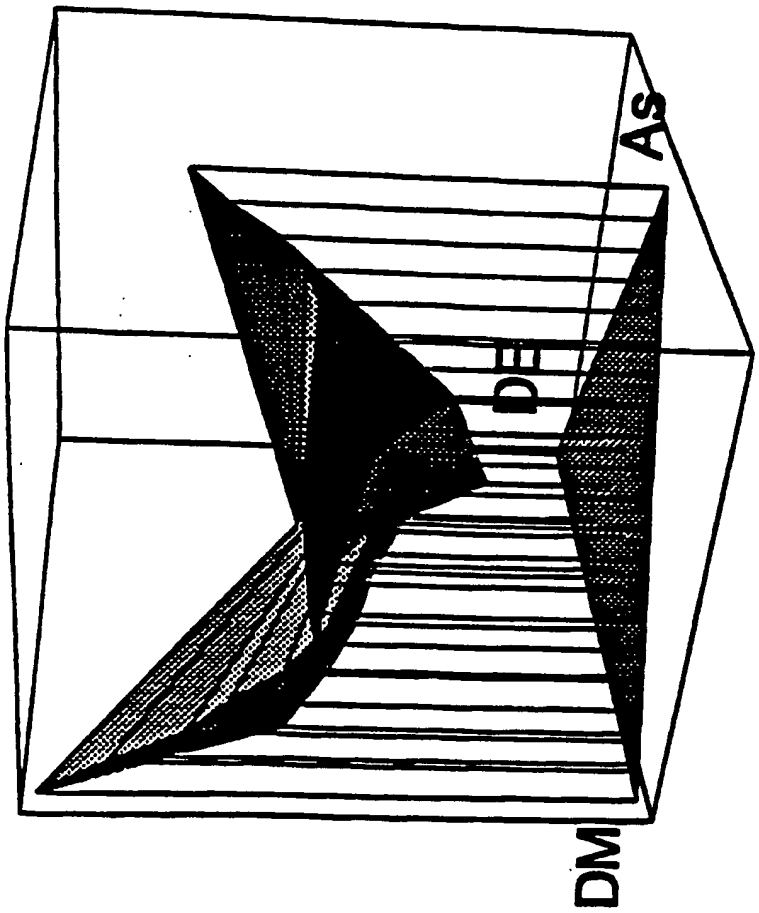


FIGURE 4C

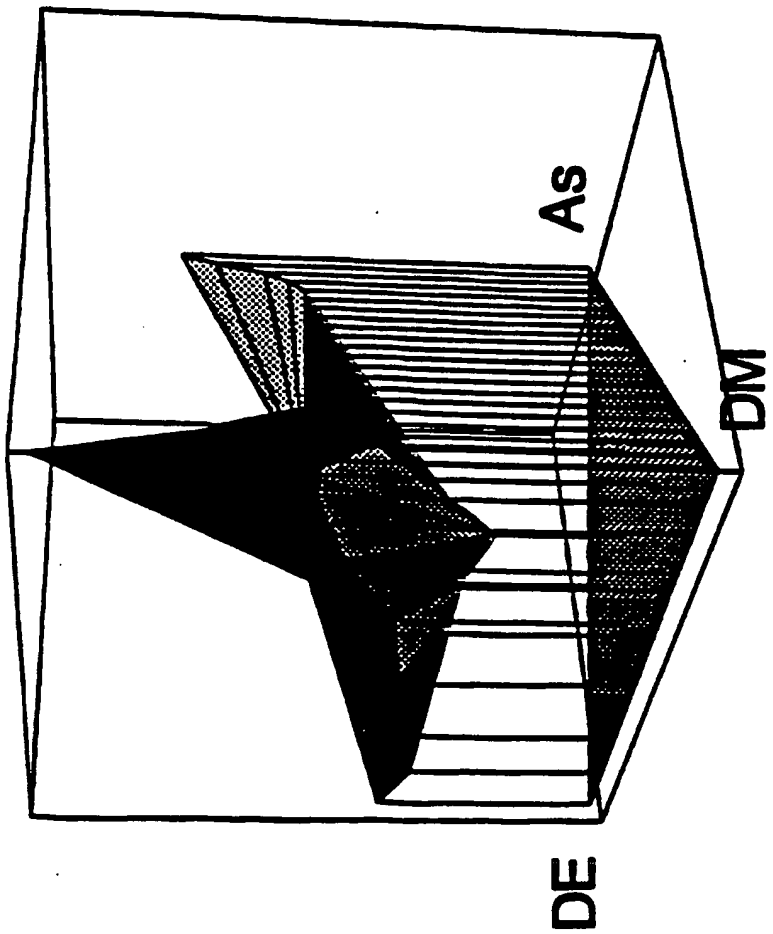


FIGURE 4D

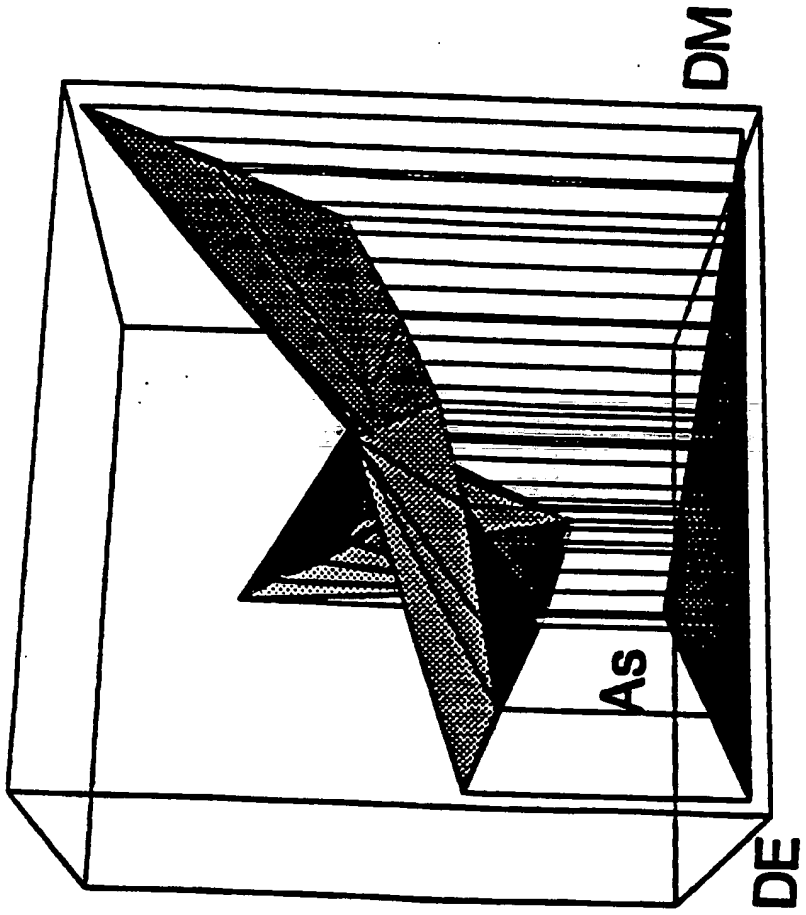


FIGURE 4E

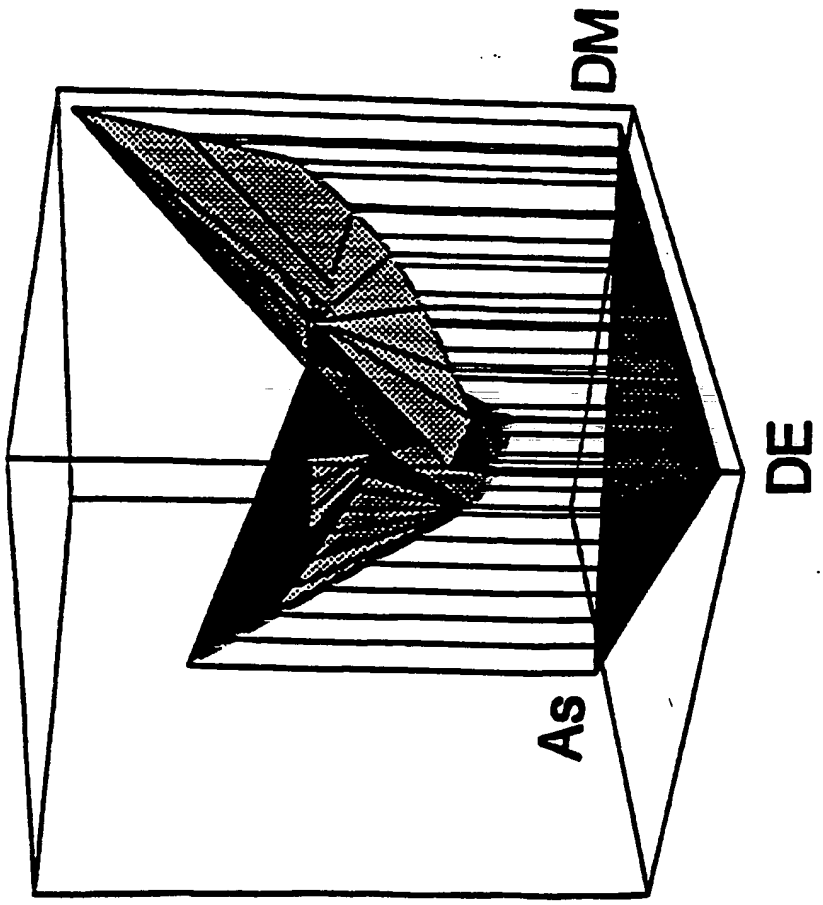


FIGURE 4F

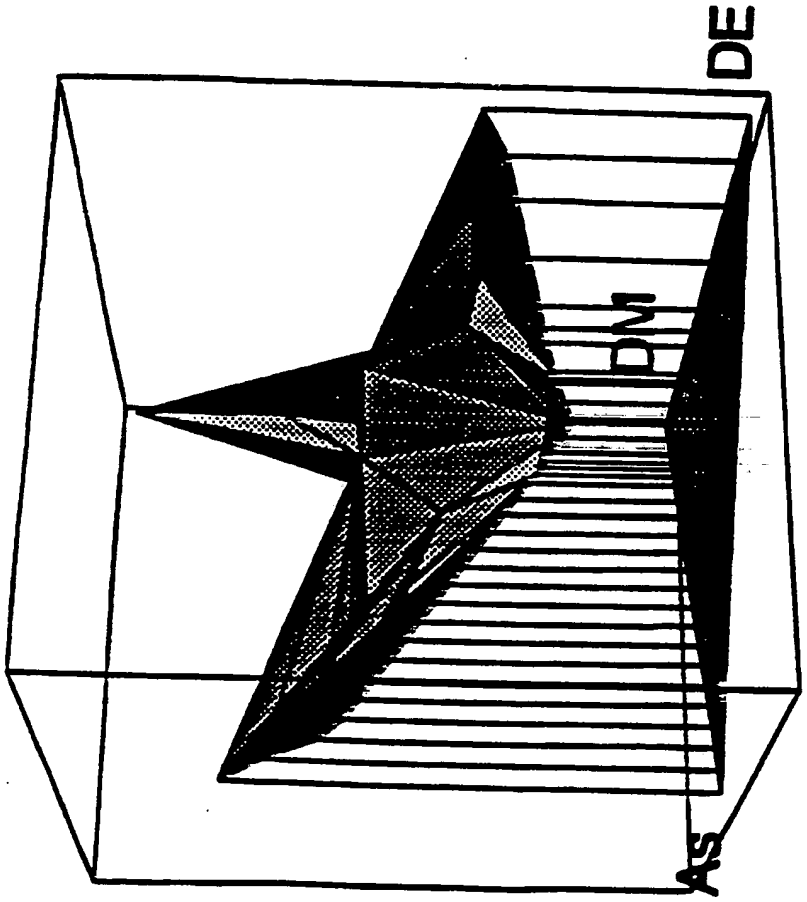


FIGURE 4G

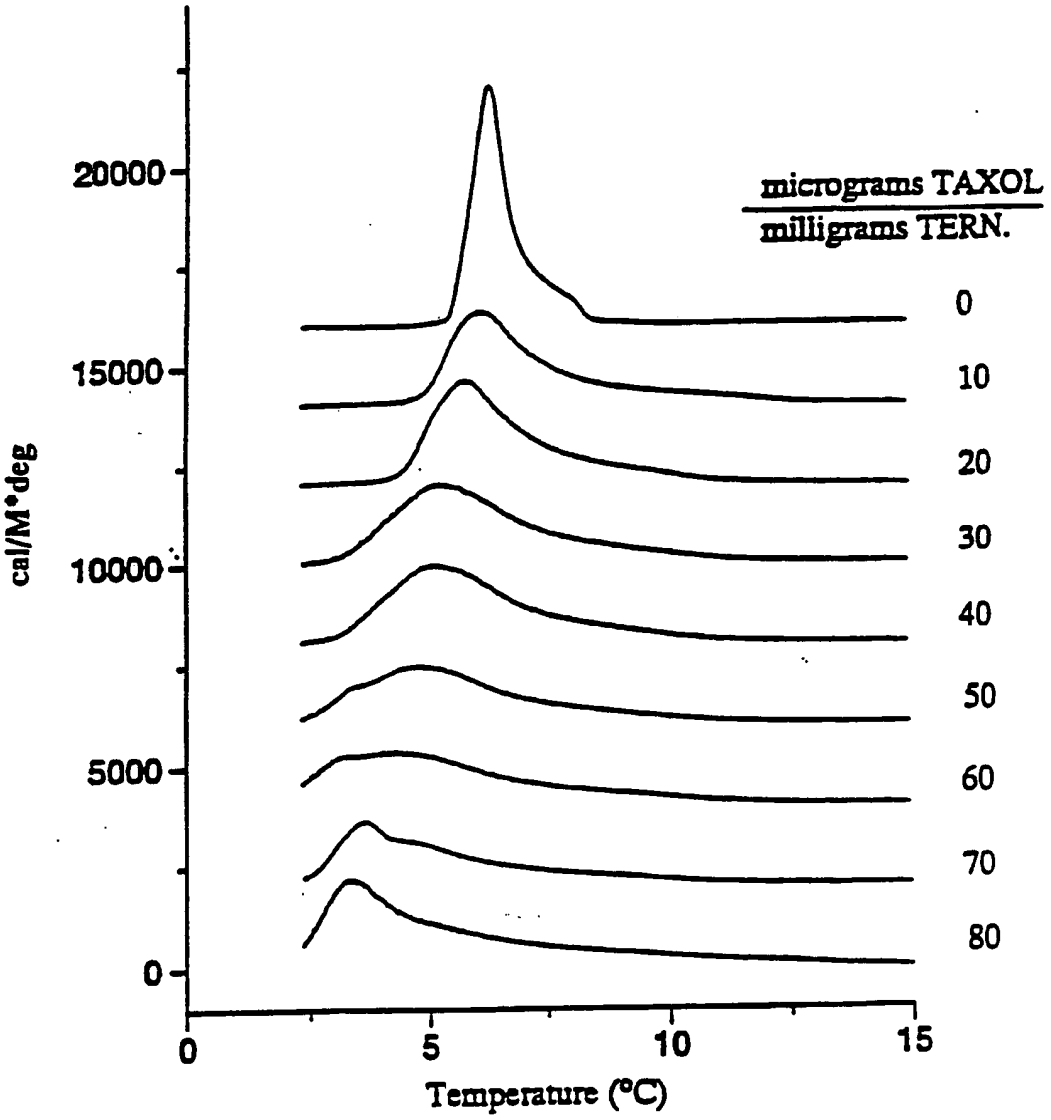

micrograms TAXOL
milligrams TERN.
0
10
20
30
40
50
60
70
80
20000
15000
10000
5000
0
cal/M*deg
0
5
10
15
Temperature (°C)

Fig. 5

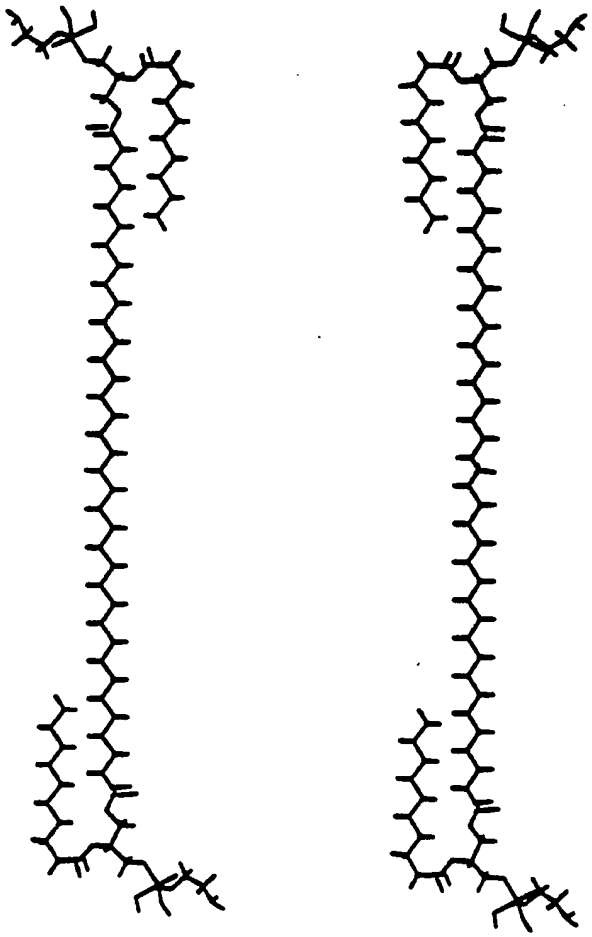


FIGURE 6

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 94/05332

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROGRESS LIPID RESEARCH, vol.27, 1988, LONDON (GB) pages 325 - 359 J.L. SLATER ET AL. 'interdigitated bilayer membranes' cited in the application see page 328 see page 352 - page 353, paragraph C1. ---	1-35
A	US,A,4 921 706 (ROBERTS ET AL.) 1 May 1990 see the whole document ---	1-35
A	EP,A,0 272 091 (VESTAR, INC.) 22 June 1988 see figure 3 see page 13 - page 15; examples 10-13 -----	1-35

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 1994	23. 09. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Benz, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US94/05332

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: ALTHOUGH CLAIMS 24-32 ARE DIRECTED TO A METHOD OF TREATMENT OF THE HUMAN/ANIMAL BODY THE SEARCH HAS BEEN CARRIED OUT AND BASED ON THE ALLEGED EFFECTS OF THE COMPOSITION.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna ul Application No
PCT/US 94/05332

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4921706	01-05-90	CA-A-	1267842	17-04-90
EP-A-0272091	22-06-88	AU-B-	608264	28-03-91
		AU-A-	8249487	16-06-88
		CA-A-	1319614	29-06-93
		DE-A-	3786005	01-07-93
		JP-A-	63233915	29-09-88
		US-A-	5320906	14-06-94